



The influence of heterotrophy and flow on
calcification of the cold-water coral
Desmophyllum dianthus

Diploma thesis

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Index

List of figures	3
List of tables	4
List of abbreviations	5
Abstract	6
Kurzfassung	7
1 Introduction	8
1.1 <i>Desmophyllum dianthus</i>	9
1.2 Anatomy and growth of <i>Desmophyllum dianthus</i>	11
1.3 Parameters influencing growth	14
1.4 Working strategy and goals	15
2 Material and Methods	17
2.1 Study area	17
2.2 Sampling and preparation of <i>Desmophyllum dianthus</i>	19
2.3 Experimental designs	19
2.3.1 Feeding experiment	19
2.3.1.1 Plankton collection	20
2.3.2 Short-term experiment	21
2.3.2.1 Cultivation setup	21
2.3.3 Long-term experiment	22
2.3.3.1 Cultivation setup	23
2.3.3.2 Manipulated parameters	24
2.3.3.3 Monitored water parameters	26
2.4 Measurements	27
2.4.1 Feeding rates	27
2.4.2 Short-term calcification rates	28
2.4.2.1 Buoyancy Weight Technique	28

2.4.3 Long-term calcification rates	29
2.4.3.1 Buoyancy Weight Technique	30
2.4.3.2 Total Alkalinity Anomaly Technique	30
2.4.4 Long-term respiration rates	32
2.4.5 Normalization of physiological data	33
2.5 Statistical analyses	34
3 Results	35
3.1 Feeding Experiment	35
3.2 Short-term calcification rates	37
3.3 Long-term calcification rates	38
3.4 Long-term changes in metabolic activity	41
3.5 Relationship between metabolic activity and mass increase	43
4 Discussion	44
4.1 Feeding of <i>Desmophyllum dianthus</i>	44
4.2 Metabolic activity	47
4.3 Relationship between metabolic activity and mass increase	50
4.4 Calcification of <i>Desmophyllum dianthus</i>	52
4.4.1 Short-term calcification rates	52
4.4.2 Long-term calcification rates	55
4.4.3 Methodological considerations	58
5 Conclusion	61
Acknowledgements	63
References	64
Appendix	81
Selbständigkeitserklärung	95

List of figures

Figure 1.1	Overhang in Comau fjord, Chile, with characteristic <i>D. dianthus</i> Aggregation (Försterra & Häussermann 2005)	10
Figure 1.2	Schematic section of the coral histology with detailed illustration of the site of calcification and potential ion pathways (modified after Allemand et al. 2011)	12
Figure 2.1	Comau Fjord in northern Patagonia and geographic locations of sampling sites	18
Figure 2.2	Schematic illustration of a coral holder and downwards orientated polyps (modified after Jantzen et al. submitted b)	21
Figure 2.3	Schematic drawing of re-circulating flow chamber used for long-term cultivation experiments	24
Figure 3.1	Capture rates of <i>D. dianthus</i> referred to three different flow speeds	36
Figure 3.2	Short-term calcification rates of <i>D. dianthus</i> specimens grown <i>in situ</i> and in the laboratory	37
Figure 3.3	Total mass increase during the long-term cultivation experiment	38
Figure 3.4	Long-term changes in calcification rate	40
Figure 3.5	Long-term changes in metabolic activity	41
Figure 3.6	Relationship between respiration rates and mass increase of <i>D. dianthus</i>	43

List of tables

Table 1	Systematic classification of <i>Desmophyllum dianthus</i>	9
Table 2	Growth rates of <i>D. dianthus</i> examined by different methods	13
Table 3	Experimental design of the feeding experiment	20
Table 4	Experimental design of the long-term cultivation experiment	22
Table 5	Nutrient concentrations of aquarium systems during long-term cultivation	27
Table 6	Summarized results of respiration and calcification rates of the different treatments during the long-term cultivation experiments	42

List of abbreviations

h	Hour
C	Carbon
O₂	Oxygen in its molecular form
CO₂	Carbon dioxide
CaCO₃	Calcium carbonate
TA	Total alkalinity
DIC	Dissolved inorganic carbon
R_T	Respiration rate
DBL	Diffusive boundary layer
ATP	Adenosintriphosphat
SD	Standard deviation
SEM	Standard error of the mean

Abstract

The solitary cold-water coral *Desmophyllum dianthus*, commonly known as deep-sea species, shows an unusual shallow (< 25 m) and dense occurrence (up to 1500 individuals m²) in the northern Patagonian fjord region. This study investigated these shallow-living *D. dianthus* specimens under exposure to their natural ecoenvironmental gradients and examined the influence of food concentration and flow regime on calcification rates and metabolic activity.

Buoyancy weight measurements revealed the highest *in situ* short-term (~ 2 weeks) calcification rates known for *D. dianthus* so far with a mass increase of 5.44 ± 3.45 mg CaCO₃ cm⁻² d⁻¹ and a corresponding weight gain of 0.25 ± 0.18 % d⁻¹. Corals that were simultaneously maintained under flow-through conditions on-site showed significantly lower calcification rates of 1.86 ± 1.37 mg CaCO₃ cm⁻² d⁻¹ and 0.09 ± 0.08 % d⁻¹. Capture rates and prey-size selectivity was ascertained by incubation experiments under supply of their natural food spectrum. Corals captured most (55 ± 16 prey items cm⁻² h⁻¹) under flow speeds of 1.7 cm s⁻¹ and showed declining trends towards stagnant and stronger flow regimes. Prey items greater 1000 µm covered approximately half of their diet.

Food availability appeared to significantly influence key physiological processes of *D. dianthus*. This was examined via laboratory long-term experiments (3 month) with corals maintained in re-circulating flow-chambers. Calcification and respiration rates increased most notably under high food and high flow conditions.

The present thesis extended the current knowledge about these shallow-living cold-water corals by investigating their feeding ecology, metabolic fitness and growth behavior under varied environmental parameters.

Kurzfassung

Die solitäre Kaltwasserkoralle *Desmophyllum dianthus*, im Allgemeinen bekannt als Tiefsee-Spezies, weist ungewöhnlich flache (< 25 m) und dichte (bis zu 1500 Individuen m²) Vorkommen in den Fjordregionen des nördlichen Patagoniens auf. Die vorliegende Studie hat diese flach lebenden Exemplare innerhalb ihres natürlichen Ökosystems untersucht und den Einfluss von Futterkonzentration und Strömungsstärke auf deren Kalzifizierungsraten und metabolische Aktivität bestimmt.

Schwimmend-Gewicht Wägungen wiesen die höchsten *in situ* Kurzzeit-Kalzifizierungsraten (~ 2 Wochen) auf, die bisher für *D. dianthus* entdeckt wurden. Sie zeigten einen Massenzuwachs von 5.44 ± 3.45 mg CaCO₃ pro cm² pro Tag und eine Gewichtszunahme von 0.25 ± 0.18 % pro Tag. Kalzifizierungsraten von Korallen die zeitgleich unter Durchflussbedingungen vor Ort gehältert wurden waren mit einem Massenzuwachs von 1.86 ± 1.37 mg CaCO₃ pro cm² pro Tag und einer Gewichtszunahme von 0.09 ± 0.08 % pro Tag signifikant geringer. Fraßraten und Selektivität bezüglich Beutegröße wurden mit Hilfe von Inkubationsexperimenten und unter Zugabe ihres natürlichen Nahrungsspektrums ermittelt. Die höchsten Fraßraten erfolgten unter einer Strömungsgeschwindigkeit von 1.7 cm s^{-1} , mit abnehmendem Trend in Richtung stagnierenden und höheren Strömungsbedingungen. Ungefähr die Hälfte ihrer aufgenommenen Nahrung bestand aus Beuteorganismen mit einer Größe von über 1000 µm.

Nahrungsverfügbarkeit erwies sich als bedeutend für den Ablauf physiologischer Prozesses. Dieses wurde durch Langzeitexperimente (3 Monate) ermittelt, die unter anderem auch die Hälterung der Korallen in Strömungskammern beinhalteten. Der stärkste Anstieg von Kalzifizierungs- und Respirationsraten wurde unter hohen Futter- und Strömungsbedingungen verzeichnet.

Diese Studie trug dazu dabei das Wissen über diese flach lebende Kaltwasserkorallenart zu erweitern indem sie deren Nahrungsökologie, die metabolische Fitness und das Wachstumsverhalten unter sich verändernden Umweltparametern untersuchte.

1 Introduction

Corals are often associated with warm and bright tropical waters, but more than 50 % of the approximately 5100 coral species inhabit deep and cold-water habitats (Roberts et al. 2009). The existence of these cold-water corals has been known for centuries but observations and studies of their habitats began only recently in the last decades facilitated by advanced deep-sea exploring devices (Freiwald et al. 2004). Cold-water corals are widely distributed and can be found from the northernmost location in the southwestern Barents Sea (Fosså et al. 2000) to the Antarctic Shelf Region (Cairns 1982). They are mainly restricted to edges of continental shelves, offshore sub-marine banks and jagged topographies such as canyons and fjords (Freiwald et al. 2004). High productive sea-surface waters, ambient current strength and elevated hard substrata for settlement seem to be key parameters influencing their occurrence (van Rooij et al. 2003, Kiriakoulakis et al. 2004). In contrast to their tropical counterparts cold-water corals thrive in depths below the euphotic zone typically lacking photoautotrophic symbionts (Freiwald et al. 2004). Hence, the relatively low growth rates recorded for cold-water corals (e.g. Adkins et al. 2004, Gass & Roberts 2006, Orejas et al. 2011a) can be referred to absence of these energy-delivering symbionts and to lowered metabolic activity in cold habitats.

In general, there are three important cold-water coral taxa: the hydrocoralli, the octocorallia including soft corals and gorgonians and finally the hexacorallia, with the orders of Zoanthidae, Antipatharia and Scleractinia (Cairns 2007). Scleractinia, also known as stony corals, are mainly characterized by their robust calcium carbonate exoskeleton (Cairns 1994). The branching and framework-building species are ecosystem engineers as they form three-dimensional complex reef structures and carbonate mounds which provide diverse deep-sea habitats (e.g. Mortenson et al. 2001, Roberts et al. 2006). These extensive cold-water coral reefs, most frequent distributed along the Norwegian continental margin, are also often accompanied by solitary scleractinian corals. Although solitary cold-water scleractinia do not occur in these massive colonial structures, they represent the absolute majority with 74 % of species (Cairns 2007).

1.1 *Desmophyllum dianthus*

The solitary cold-water scleractinia *Desmophyllum dianthus* (syn. *cristagalli*) belongs to the family of caryophyllidae (Table 1) and was first described by E.J.C. Esper in 1794.

Table 1: Systematic classification of *D. dianthus*

Phylum:	Cnidaria
Class:	Anthozoa
Subclass:	Hexacorallia
Order:	Scleractinia
Family:	Caryophyllidae
Subfamily:	Desmophyllinae
Genus:	Desmophyllum
Species:	<i>Desmophyllum dianthus</i>

Desmophyllum dianthus is distributed throughout the world's ocean basins with records in the North (Sorauf & Jell 1977) and West Atlantic (Cogswell et al. 2009), the North Pacific (Cairns 1994), the Mediterranean (Taviani et al. 2005) as well as at the Chilean (Försterra et al. 2005), South African and Australian coasts (Zibrowius 1980a). The bathymetric distribution of *D. dianthus* is located between 35 – 2460 m water depth (Försterra et al. 2005), although Risk et al. (2002) report extensions down to bathyal depth of 4000 m. Nevertheless, in the Chilean and New Zealand fjord regions it was documented within the euphotic zone where it occurs in dense aggregations (Försterra & Häussermann 2001, 2003, Cairns et al. 1995, 2005, McCulloch et al. 2005). A particularly unusual shallow (< 25 m) and dense occurrence (up to 1500 specimen m²) of *D. dianthus* was described for the Comau Fjord, located within the northern part of the austral Chilean fjord system (Försterra & Häussermann 2005). Here, corals were found mainly on overhangs and rock ledges where the downward orientated polyps grow underneath (Fig. 1.1). This characteristic growth pattern may be caused by the high sediment input via rivers in this fjord systems and the sensitivity of tentacle feeders against it (Rogers 1990). These extensive coral banks play an important ecological role by providing structural habitat to a diverse benthic fjord community. Epibiontic organisms such as sponges, foraminiferas, hydroids and polychaetes often intensively

colonize the bare skeleton of a corallum. This part can also be used as settle-ground by *D. dianthus* larvae which results in the formation of pseudo-colonies containing up to twenty individuals (Försterra & Häussermann 2003). Even though *D. dianthus* is known as azooxhantellate organism, it shows an unusual symbiotic relationship to endolithic greenalgae and cyanobacteria located underneath the coral tissue. Försterra et al. (2005) hypothesize that the endoliths are protected from sedimentation and grazers, whereas the corals gain phototrophic assimilates for skeletal mineralization.



Fig. 1.1: Overhang in Comau fjord, Chile, with characteristic *D. dianthus* aggregation
(Försterra & Häussermann 2005)

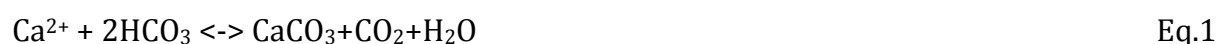
Chilean cold-water corals, like all other cold-water coral bioherms, are threatened by variety of anthropogenic impacts. Beside the global dimension of acidifying oceans (e.g. Riebesell et al. 2000, Orr et al. 2005), warming of deep-water masses (Barnett et al. 2001) and disturbance of deep sea habitats via commercial fishery (Fosså et al. 2002), Chilean fjord ecosystems are particularly endangered by a rapid developing aquaculture (Häussermann & Försterra 2007). Especially in these semi-closed fjord environments extensive aquaculture and resultant nutrient pollution causes an enhanced eutrophication and corresponding oxygen depletion of bottom waters – presumably a serious threat for local *D. dianthus* communities.

1.2 Anatomy and growth of *Desmophyllum dianthus*

Their robust aragonitic skeleton is symmetrically arranged with hexameral mesenteries and septa crucial for the stability of the calyx (Daly et al. 2003). The calyx can be roughly separated in an apical part covered with tissue and a basal part composed of bare skeleton without tissue (Försterra et al. 2005). The polyp tissue itself consists of two different layers: the epidermis and the gastrodermis, separated by a thin extracellular matrix of collagen fibres called mesogloea (Fig. 1.2). These tissue layers can be divided into an oral and aboral part with the coelenteric cavity in between (Allemand et al. 2004). This coelenteric cavity, also termed coelenteron, is responsible for the nutritional exchange of the coral. Any solid wastes can be excreted whereas the tentacles, arranged in multiple of six around its opening, help to take up food particles (Fautin & Marisca 1991). The tissue layer which faces the skeleton (aboral ectodermis) is known as the calicoblastic epithelium. These large (10 to 100 µm) calicoblastic cells contain numerous mitochondria and are considered as patterns important for calcium carbonate (CaCO₃) formation (Tambutté et al. 2007)

By now, two different hypotheses have been developed in regard to calcification mechanisms of cold-water corals and corals in general. One model (Barnes 1970) describes calcification as a physico-chemical process occurring within special pockets, where the calicoblastic ectoderm is lifted away from the skeletal surface. Constantz (1986) and Cohen & McConnaughey (2003) supported this hypothesis by expectation of an extracellular calcifying fluid between skeleton and calicoblastic cells, where carbonate crystals precipitate due to inorganic processes. Another model supposes calcium carbonate precipitation to depend on biologically-controlled mineralization processes (Mann 1983) within an organic matrix secreted by the tissue (Wheeler 1984, Allemand et al. 1998, 2004).

However, the chemical reaction of calcification can be simplified by:



The required calcium (Ca²⁺) and bicarbonate (HCO₃) ions are derived from external seawater and are delivered to the site of mineralization (Fig. 1.2). Until now it is not

known whether the transport occurs transcellular via ion carriers or paracellular via passive diffusion through cell junctions (Allemand 2011). Potential pathways and calcification mechanisms of *D. dianthus* will be discussed later on in more detail (chapter 4.3).

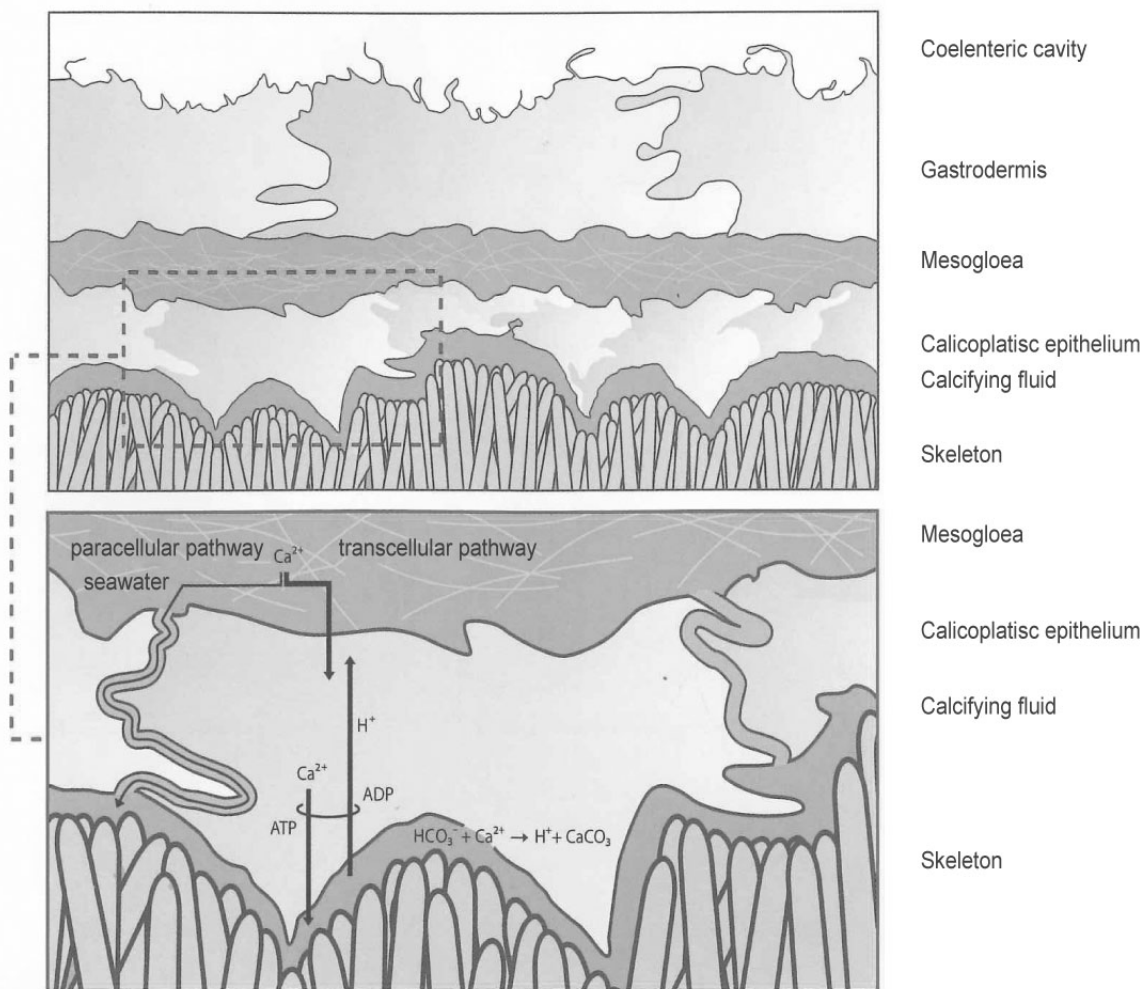


Fig. 1.2: Schematic section of the coral histology with detailed illustration of the site of calcification and potential ion pathways (modified after Allemand et al. 2011)

In general, growth can be expressed as an increase in volume, area, mass or linear extension. These different growth parameters can be identified by several methodological approaches. Beside photographic (Purser et al. 2009, Langdon et al. 2010, Kupprat 2011) and direct measurements (Gass & Roberts 2006, Orejas et al. 2008, Brooke & Young 2009) for detection of linear extension, alternatively the staining

technique with an Alizarin red dye can be applied (Lamberts. 1978, Dodge 1984, Brooke & Young 2009). Particularly for *D. dianthus* also isotopes and isotope ratios were used in several previous studies for estimations of linear extension rates (Cheng et al. 2000, Risk et al. 2002, Adkins et al. 2004). Jokiel et al. (1978) established the buoyant weight technique which measures skeleton weight and density. The uptake of carbonate ions can be used for the determination of calcification rates by the total alkalinity (TA) anomaly technique (Smith & Key 1975). Furthermore, calcium deposition can be examined by labeling techniques resolving the incorporation of ^{45}Ca (Maier et al. 2009, Langdon et al. 2010). Estimated growth rates of *D. dianthus* and applied methods are summarized in table 2.

Table 2: Growth rates of *D. dianthus*, examined by different methods.

Origin	Depth (m)	Method	Growth location	Growth ($\mu\text{mol CaCO}_3$ g^{-1} dry mass d^{-1})	Weight increase (% d^{-1})	Linear extension (mm yr^{-1})	Reference
Patatagonia, Chile	22	observations	<i>in situ</i>	n.a.	n.a.	2.3 length 1.6 diameter	Försterra & Häussermann 2003
Mediterranean Sea	425-585	TA technique	laboratory	38 ± 14	0.1-0.3	n.a.	Naumann et al. 2011
Mediterranean Sea	300	buoyant weight	laboratory	n.a.	$0.06 \pm$ 0.03	n.a.	Orejas et al. 2011
Pacific & Atlantic	420– 2200	U/Th dating	<i>in situ</i>	n.a.	n.a.	0.1 – 3.1	Cheng et al. 2000
n.a.	n.a.	U/Th and ^{14}C dating, verified by observations	<i>in situ</i>	n.a.	n.a.	0.5 – 1.0	Risk et al. 2002
South Pacific	Deep-sea	$^{226}\text{Ra}/^{210}\text{Pb}$ radio- nucleotide decay	<i>in situ</i>	n.a.	n.a.	0.5 – 2.0	Adkins et al. 2004

1.3 Parameters influencing growth

There are many different biotic and abiotic factors influencing coral growth, such as the availability of food and nutrients, the water temperature, the ocean chemistry, and the hydrography of the habitat (Lough & Barnes 2000, Guinotte et al. 2006, Davies et al. 2008, Dullo et al. 2008). The present study focuses on flow regime and coral nutrition.

Flow regime

Water flow affects coral growth in various ways: it influences the food supply, removes waste products, reduces sediment deposition and is crucial in terms of gas exchange (Roberts et al. 2009).

Food supply of corals, as of all other sessile tentacle feeders, mainly depends on ambient current strength (Eckman & Duggins 1993). The enhancement of particle capture and ingestion rates with raising flow increases the amount of energy available for growth mechanisms. Also the thickness of the tissues covering boundary layer is flow dependent and bias the removal of harmful by-products and gas exchange (Patterson et al. 1991b, Shashar et al. 1993). Respiration and corresponding oxygen consumption in turn are necessary for the intracellular energy production (Willmer et al. 2008).

The importance of an optimal exposure to flow of *D. dianthus* is indicated by their characteristic growth forms within aggregations (Försterra & Häussermann 2003). *Desmophyllum dianthus* can be mainly distinguished in short shaped individuals with massive skeleton structures and elongated corallums with delicated walls and small calicular diameters. The latter described intensified length growth is only visible in the centers of coral accumulations and seems to optimize the polyp's exposure to flow.

Heterotrophy

In general, scleractinian corals are able to utilize a variety of food sources. They can compensate their energy demand through dissolved (Al-Moghrabi et al. 1993, Grover et al. 2008) and particulate organic matter (Anthony 1999, Anthony & Fabricius 2000), phytoplankton (Fabricius 1998) zooplankton (Sebens et al. 1996, 2003, Ferrier- Pagès et al. 2003, Palardy et al. 2005) and even through chemosynthetic bacteria (Hovland & Risk 2003), discovered in some cold-seep species.

As cold-water corals typically lack energy delivered by zooxanthella, they are supposed to depend on heterotrophy and the concluding uptake of zooplankton and suspended particulate organic matter (Freiwald et al. 2004). Their potential dietary has been investigated by analyses of the bulk fatty acid composition (Kiriakoulakis et al. 2005), stable isotope ratios (Duinevald et al. 2004), lipid biomarkers (Dodds et al. 2009) and by *in situ* video surveys (Tsounis et al. 2010). Some studies concluded mesozooplankton as the primarily food source (Duinevald et al. 2004, Kiriakoulakis et al. 2005) but a complete nutritional budget is not established by now.

A recently published study on *D. dianthus* (Naumann et al. 2011) gives first evidence for the trophic importance of zooplankton since the uptake of this energy-rich food source significantly increased calcification rates.

1.4 Working strategy and goals

The current knowledge about the ecology and physiology of the cold-water coral *D. dianthus* is still very limited. Hence, their shallow occurrence within Comau Fjord (Patagonia, Chile) provided a unique opportunity to investigate these organisms which usually thrive in hardly accessible depths below the euphotic zone. Shallow living *D. dianthus* specimens face strong environmental variations naturally caused by tidal fluctuations, turbulent mixing processes and seasonal changes in plankton availability. The aim of the present study was therefore to evaluate the influence of nutrition and flow speed on coral growth and general metabolic fitness.

A set of pre-experiments conducted during an expedition to the Comau Fjord provided insight into organism responses under exposure to their natural ecoenvironmental gradients.

- *In situ* short-term growth rates were determined and served as a reference for growth rates of corals maintained in aquaria systems
- Feeding rates and preferences in prey-size and -species were assessed by supply of their natural food spectrum and under varied flow conditions

The obtained results and additional measurements of physico-chemical water properties were finally used to adjust the maintenance conditions for subsequent laboratory long-term experiments.

These long-term cultivation experiments were carried out at aquaria facility available at the Alfred Wegener Institute for Polar and Marine Research (AWI), Bremerhaven. They were designed to determine combined effects of nutritional status and flow regime on calcification and metabolic activity of *D. dianthus* and addressed the following hypotheses:

- (1) Food enhancement increases calcification rates and general metabolic fitness of *D. dianthus*

H₀: food enhancement does not increase calcification rates and coral fitness

- (2) Raising flow increases metabolic activity of *D. dianthus* which in turn also promotes calcification processes

H₀: raising flow has no positive effect on metabolic activity and calcification

2 Material and Methods

2.1 Study area

The austral Chilean fjord region extends from 41.47 °S to 56.00 °S and represents a complex ecosystem with high benthic species diversity (Försterra et al. 2005). The 34.3 km long Comau Fjord is located in the northern part of Chilean Patagonia and is north-south orientated along the 72.30 °W longitude (see Fig. 2.1). Its depth decreases from ~ 600 m at the mouth to less than 50 m near the head, where two rivers discharge (Försterra & Häussermann 2010). These rivers, fed by melting glaciers and precipitation supply a constant freshwater influx into the fjord causing a strong stratified water column with a low salinity layer at the top (halocline located within the upper ~ 12 m) and marine conditions below (Jantzen et al. submitted a). Vertical oxygen, pH and temperature gradients show a gradual decrease with depth. The surface layer is well-oxygenated (5 - 8 mL L⁻¹) with a pH of 8.0 - 8.3 and a temperature of ~ 12 °C in contrast to the deep layer (below 75 m) with 3 - 4 mL L⁻¹ of dissolved oxygen, a pH of 7.4 - 7.7 and a temperature of ~ 10.7 °C (Silva 2008, Jantzen et al. submitted a). Furthermore, rivers transport large amounts of organic and inorganic matter into the fjord systems which accumulate in the deep. Silva et al. (2008) found low nutrient concentrations (0.0 - 0.08 µmol phosphate, 1.2 - 2.4 µmol nitrate, < 0.5 µmol ammonium) within the upper layer compared to those found within the deep layers (0 - 8 µmol phosphate, 12 - 24 µmol nitrate, 0.5 - 2.5 µmol ammonium). The continuous exchange of intermediate water masses between fjord Comau and the adjacent Gulf of Ancud might additionally effect the fjord environment. The Gulf of Ancud is characterized by a high primary production (Iriarte et al. 2007) and according biomass of copepods and euphausiacea (González et al. 2010) which also suggests high seasonal plankton concentrations within the fjord environment. Flow conditions within Comau Fjord are mainly influenced by tidal circulations and the local topography. Measurements, conducted according to Hart et al. (2002), revealed at the fjord's mouth a greater range of flow speed (*Liliguapi*: 0.5 - 11 cm s⁻¹) as inside the fjord (*Cross-Huinay*: 3.1 - 5.4 cm s⁻¹).

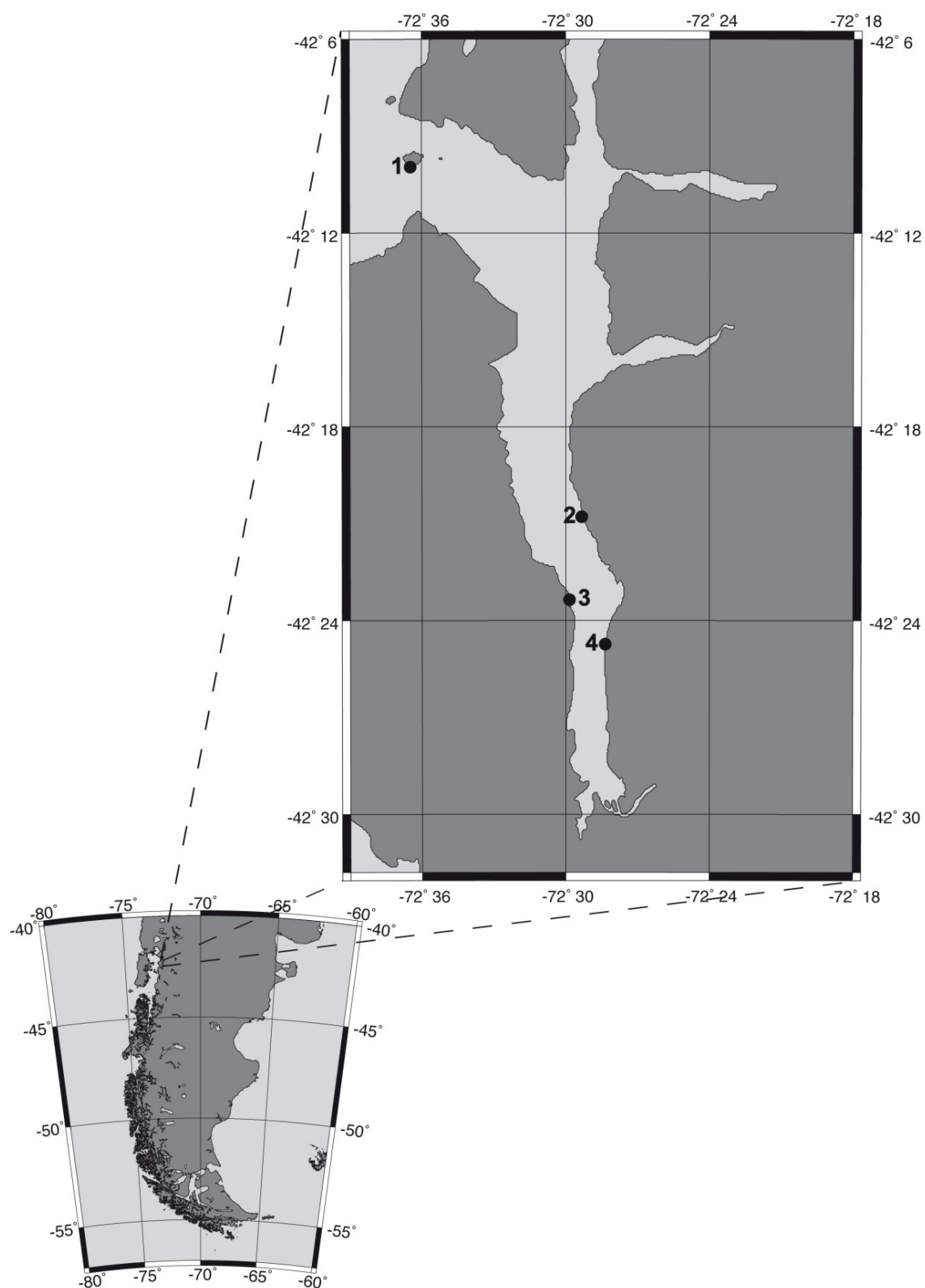


Fig. 2.1: Comau Fjord in northern Patagonia and geographic locations of sampling sites, numbered from the mouth towards the head as followed: 1: *Liliguapi*, 2: *Swall*, 3: *Cross-Huinay*, 4: *Punta Gruesa*.

2.2 Sampling and preparation of *Desmophyllum dianthus*

Sixty living *D. dianthus* individuals were collected in February 2011 at two different sites within Comau fjord: 30 corals at *Liliguapi*, a small island located at the mouth and the other 30 at *Cross-Huinay* in the central part of the fjord. Corals used for long-term experiments in Bremerhaven also included samples collected previously in 2010 at *Punta Gruesa*, *Swall* and *Cross-Huinay* (Fig. 2.1).

Sampling was carried out in 20 m water depths via SCUBA diving. The corals were chiseled off, transported back on board and stored in a thermo-box filled with water of the sampling depth which was pre-obtained using a Niskin Bottle. Within 1 hour after collection corals were brought back to the laboratory. There, bare skeleton was removed with a submerged diamond blade and straightened fracture zone was sealed with the same cyano-acrylate gel (UHU Superflex Gel, without solvent) as used in the study of Jury et al. (2010). The sealed area was additionally fixed to a polyethene-screw in order to replicate the *in situ* growth direction during cultivation and enable better handling of coral fragments (Fig. 2.2). Samples were left for 2 hours in a separated spill-over tank until the glue was hardened. Afterwards, they were marked, weighed (Sartorius CPA 225D-0CE; see chapter 2.4.2.1) photographed (Canon PowerShot® G11/12, resolution 10.0 megapixels) and measured in length and diameter with a plastic calliper. The volume of each coral was determined using water displacement measurements within a 50 mL Falcon Tube or a 200 mL glass beaker, depending on coral size.

2.3 Experimental designs

2.3.1 Feeding experiment

Feeding rates of *D. dianthus* were assessed at field station *Fundacion Huinay* under exposure to their natural food spectrum and varied flow conditions. Four replicated feeding experiments per flow treatment (0 cm s^{-1} , 1.7 cm s^{-1} , 4.3 cm s^{-1}) were performed, separated into two periods a day with three simultaneously running incubations (Table 3). Feeding experiments were carried out at night considering the natural biological rhythm as corals are assumed to expand their tentacles mainly nocturnally (Levy et al.

2001) to actively feed on diurnal migrating zooplankton (Heidelberg et al. 2004). Control incubations without corals (blanks) were conducted in order to estimate the amount of internal grazing.

Table 3: Design of the feeding experiment. The different treatments: 0 cm s⁻¹, 1.7 cm s⁻¹, 4.3 cm s⁻¹ (each with 4 replicates) and corresponding controls (each with 2 replicates) were separated into 6 runs over a period of 3 days. Each run consisted of 3 simultaneously running incubations.

Day	Run	Treatment					
		0 cm s ⁻¹		1.7 cm s ⁻¹		4.3 cm s ⁻¹	
		corals	blank	corals	blank	corals	blank
1	I		1x	2x			
	II	1x			2x		
2	I		1x			2x	
	II	1x		2x			
3	I	1x					2x
	II	1x				2x	

2.3.1.1 Plankton collection

Each night (20 – 21 pm) prior to experiments a 15 min plankton tow was conducted within the fjord using a 0.5 m diameter net with a mesh size of 500 µm. The net was passed with a continuous velocity of 12 m min⁻¹ through the water column sieving a total volume of approximately 35 m³. A bathometer fastened to the weights of the net reported a maximum depth of 73 m. Caught plankton was transferred to a 10 L bucket which was filled with fjord water from 30 m water depth and transported immediately back to the laboratory. There, the actively swimming upper portion of the haul was separated using 50 µm gauze and concentrated into a 1 L volumetric flask. Five 50 mL sub-samples were taken with a large syringe steered homogeneously through the water body to determine the zooplankton composition and abundance on each experimental

day. The general plankton concentration varied within the 4 days of experiment depending on natural changing plankton succession of the fjord environment.

2.3.2 Short-term experiment

Short-term calcification rates of corals grown *in situ* were comparative examined to those maintained in the laboratory of field station *Fundación Huinay*.

Therefore, 10 individuals of each sampling site (*Liliguapi* and *Cross-Huinay*) were returned after weighing to their natural environment whereas the other 10 were transferred into cultivation facilities. All corals were screwed in customized holders (Fig. 2.2), which were either attached to the frame of the aquaria tanks or fixed via anchor-dowels to the basaltic substratum of the respective sampling site. Corals were re-weighed after 12 - 14 days.

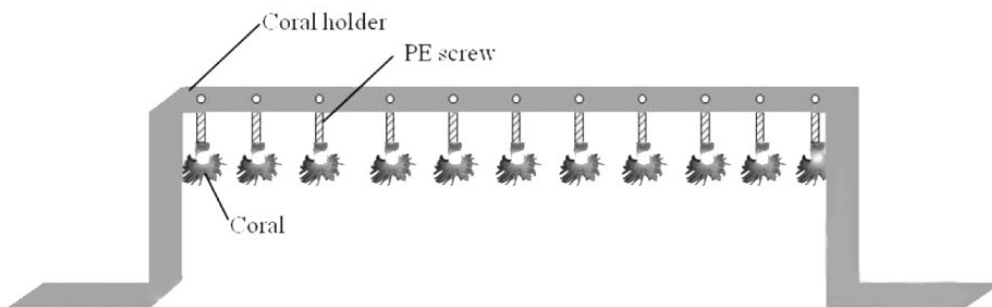


Fig. 2.2: Schematic illustration of a coral holder and downwards orientated polyps.

Modified after Jantzen et al. (submitted b).

2.3.2.1 Cultivation setup

Twenty *D. dianthus* specimens were cultivated in a flow-through aquarium system (10 individuals per 24.5 L tank), which was continuously supplied with pre-filtered (fleece filters) fjord water at a renewal rate of $\sim 1.25 \text{ L min}^{-1}$. This water was pumped from $\sim 20 \text{ m}$ water depth and provided water conditions close to *in situ*. Water parameters

(Hach HQ 40d multi logger) revealed mean values of: temperature = 12.1 ± 0.3 °C, salinity = 32.8 ± 0.9 , pH = 7.98 ± 0.07 and an oxygen concentration of 10.81 ± 0.62 mg L⁻¹. Water movement inside the tanks was enhanced by aquarium pumps (Eheim 2006) circulating at 3 L min⁻¹. As pre-filtration prevented the delivery of zooplankton abundant in seawater, corals were fed twice a week with plankton caught within the fjord (50 µm net).

2.3.3 Long-term experiment

To evaluate the cross-effect of flow and feeding on growth rates and fitness of *D. dianthus*, a two-factorial (2 x 2) long-term cultivation experiment was designed. Two levels of flow were combined with two levels of food (Table 4), which in turn resulted in four different treatments run over a period of 3 months. One treatment was represented by one flow chamber and therein located coral specimen (n = 9), grouped consistently according to size and residence time within maintenance facilities (Expedition 2010, 2011).

Table 4: Experimental design of the long-term cultivation experiments with four treatments (I – IV), differing in flow speed (mean \pm SD) and food status. Chambers of treatments with equal food status were set together in one aquarium system.

Aquarium system	Treatment	Flow speed (cm s ⁻¹)	Food status
1	I	2 ± 0.2	starved
1	II	5 ± 0.6	starved
2	III	2 ± 0.2	fed
2	IV	5 ± 0.6	fed

2.3.3.1 Cultivation setup

The aquaria facility at AWI were set up in a 10 °C climate chamber and consisted of two re-circulating 350 L aquarium systems, each in turn featuring two experimental tanks (27 x 26.5 x 69 cm), a maintenance tank (27 x 53 x 69 cm) and a pump sump. Each experimental tank was identically equipped with a unidirectional re-circulating flow chamber (23 x 20 x 42 cm), hosting nine corals.

Corals acclimatized for 3 weeks to the aquaria systems and for another 4 weeks to the flow chambers. Chambers were arranged with each food level referred to one aquarium system (Table 4) in order to avoid confounding effects caused by dissolved nutrients or residual food particles passing the filter. To get rid of excreted nutrients and harmful by-products and to provide sufficient trace elements needed for coral growth, water was exchanged weekly (~ 10 %) using fresh and unfiltered North Sea water. Fouling was prevented by a low light intensity and period of 36.23 PAR for 10 h, respectively. The chambers and aquaria tanks were cleaned every 4 weeks.

These flow chambers (Fig. 2.3) were self-designed and constructed as follows: The current pump (Tunze Turbelle ® nanostream ® 6015 (1.800 L h⁻¹) for weak flow conditions or the controllable Tunze 6105 (3000 – 13000 L h⁻¹) for strong flow conditions were attached to the frame of the chamber with the propeller directed outwards into the experimental tank. The water was sucked out the chamber and entered it again at the opposite site by passing the flow path. This flow path, a perforated PVC plate integrated as chamber wall, was necessary in order to get a circular flow between chamber and surrounding tank and functioned further as flow-straightener. Hence, water within the chamber was laminar transported along the corals towards the pump. Insertable coral holders were placed in the middle of each chamber with corals arranged in a staggered manner to avoid differences in flow exposure.

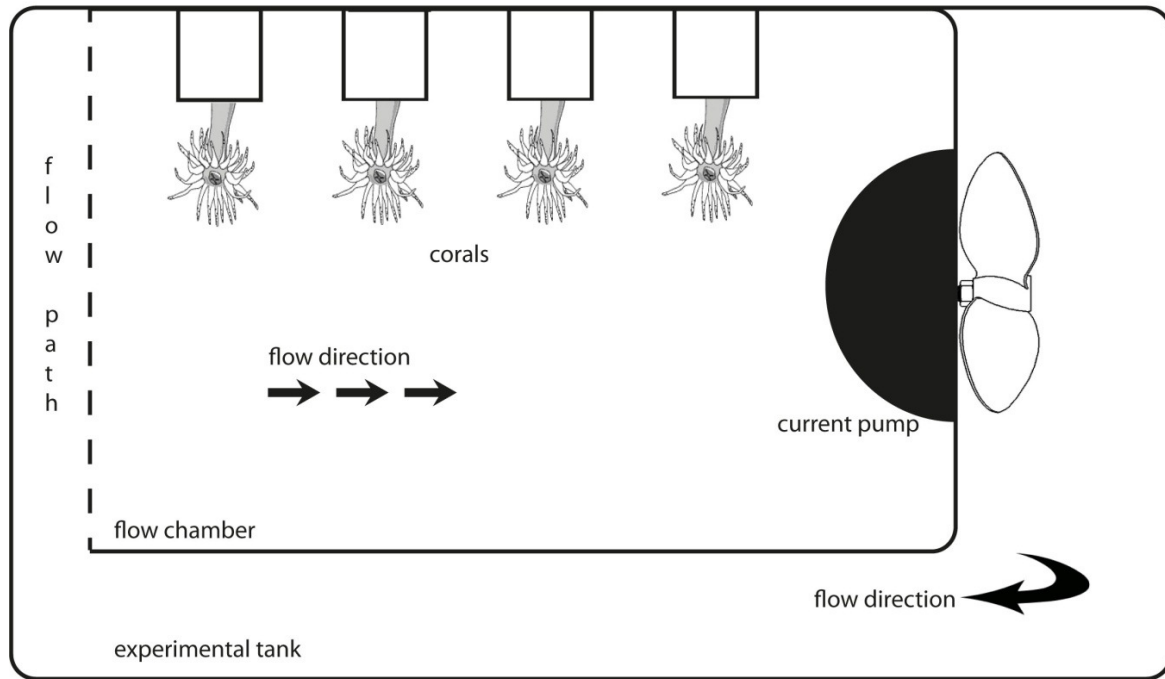


Fig. 2.3: Schematic drawing of re-circulating flow chamber (side-view) used for long-term cultivation experiments. Each chamber was placed within a 44 L tank (denoted as experimental tank) in turn connected to a 350 L aquarium system. The flow was transported homogenously through the flow path, along the corals towards the pump, driven by the propeller generated undertow. Corals ($n = 9$) were screwed to insertable holders in a staggered manner to avoid potential differences in flow exposure.

2.3.3.2 Manipulated parameters

Flow speed

Flow speeds were chosen according to flow experienced by the corals within the Comau Fjord (see chapter 2.1), with 2 cm s^{-1} and 5 cm s^{-1} representing flow conditions below and above the natural existing average (4.2 cm s^{-1}). They were adjusted using an Acoustic Doppler Velocimeter (ADV, 10 Hz, Sontek Inc.) and the applied software Horizon ADV. The ADV probe was placed at eleven different positions within two flow chambers (representative of weak and strong flow) to achieve a longitudinal chamber profile. Therefore, chambers were symmetrically separated in three different heights and widths, revealing six axes. The central crossing points of these axes were measured twice for a period of 30 seconds. Two additional measurements were taken at the

middles right and left side to also cover the latitudinal shift of current strengths. Mean flow speeds (\bar{u}) of each position were calculated using the equation:

$$\bar{U} = \sqrt{u^2 + v^2 + w^2} \quad \text{Eq.2}$$

where u, v, w are the mean velocities (cm s^{-1}) measured in three dimensions.

Food concentration

Corals were fed with frozen *Euphausia pacifica* specimen: starved corals once a week and fed corals four times a week. Individuals had to be pipetted onto expanded tentacles, the low flow speed was not sufficient to keep the food in suspension. Capture and ingestion were monitored to ensure regular food intake.

Food supply was adjusted due determined respiration rates (obtained via incubation experiments, described in chapter 2.4.3) of growing *D. dianthus* specimen. Results were used to calculate the carbon demand and needed prey items. This was mainly evaluated for starving specimens to assure a minimal food supply covering at least the respiratory carbon loss of $9.78 \pm 3.88 \mu\text{g C cm}^{-2}$ projected calyx surface h^{-1} (mean \pm SD) which was calculated according to the formula:

$$\text{Respired C} = \text{O}_2 \text{ consumed } (\text{mg cm}^{-2} \text{ h}^{-1}) \times 0.375 \text{ RQ} \times 1000 \quad \text{Eq. 3}$$

with a respiratory quotient (RQ) of 0.8 for azooxanthellate temperate anthozoans (after Muscatine 1981). The C content (μg) of the prey item *Euphausia pacifica* was determined following Ross (1982):

$$\mu\text{g C} = 0.071 + 0.324 \text{ DW} \quad \text{Eq. 4}$$

where the average dry weight (DW) of $1893.923 \mu\text{g}$ was calculated using ten *E. pacifica* specimen, dried in a oven at 60°C for 48 hours and weighed with a Sartorius 1712 MP8 balance ($< \pm 0.02 \text{ mg}$).

2.3.3.3 Monitored water parameters

Temperature, Salinity, Oxygen and pH

The physicochemical water parameters (temperature, salinity, oxygen and pH) of both systems were monitored twice a week to ensure constant conditions. A Hach multiparameter logger equipped with a CDC 401 conductivity electrode and a LDOTM 101 Optode were used to determine salinity and oxygen properties. The pH and temperature were measured with a WTW pH 3310 logger and a corresponding Blue line 14 pH electrode (Schott Instruments). This electrode was regularly three-point calibrated (Merck Certipur[®] buffer: 4.00 ± 0.01 , 7.00 ± 0.01 , 10.00 ± 0.02) to warrant the accuracy of measurements.

All parameters were kept to values found within the natural environment: temperature = 10.0 ± 1 °C, Sal = 31 ± 2 , pH = 8.0 ± 0.1 , oxygen concentration = 11.0 ± 0.5 mg L⁻¹. Evaporation was automatically compensated by a pump, which transported deionised water out of a reservoir to the pump sump once the water level fell.

Nutrients

Prior to water exchange, weakly quick tests (JBL test) of ammonium (NH₄), nitrate (NO₃), nitrite (NO₂), phosphate (PO₄), calcium (Ca) and magnesium (Mg) concentrations were used to monitor the water conditions during the long-term experiment.

Precise values were acquired once a month by additional photometrical analyses (Autoanalyzer, Alliance Instruments Evolution III) of dissolved NO_x (NO₃/NO₂) and PO₄ and fluorometrical determination (Spectrofluorophotometer, Shimadzu RF 1501) of NH₄ as described by Holmes et al. (1999). For this, 40 mL water samples of each system were taken 0.2 µm filtrated (SFCA glass fiber filter) using a 10 mL syringe (Braun Omnifix[®]). Samples were measured in duplicates to minimize deviations induced by methodical errors. Received results were averaged and revealed following nutrient conditions during long-term cultivation:

Table 5: Nutrient concentrations (mean \pm SD) of both aquarium systems during long-term cultivation

	PO₄ ($\mu\text{mol L}^{-1}$)	NO_x ($\mu\text{mol L}^{-1}$)	NH₄ ($\mu\text{mol L}^{-1}$)
System 1	0.06 \pm 0.03	6.81 \pm 4.77	0.21 \pm 0.16
System 2	0.11 \pm 0.08	2.77 \pm 2.15	1.19 \pm 1.06

2.4 Measurements

2.4.1 Feeding rates

All corals starved for 2 days before they were randomly selected and separately placed into 3 L incubation jars. The largest possible volume was chosen to minimize place limited depending capture success. The downward facing polyps were arranged edgewise within the jar to enhance their exposure to rotating plankton. Flow was generated by a magnetic stirring table (IKA ® Werke – RO 15 power) and a corresponding stir bar placed within the incubation water. The jars were left open in order to provide enough oxygen during incubations. Corals were acclimatized for half an hour to the incubation unit before prey was added. Sub-samples (100 mL per coral) of concentrated plankton were taken under continuous swiveling of the volumetric flask to achieve homogenization of prey items. Afterwards they were carefully poured into the incubation jars. Remaining plankton haul was transferred into a 6 L holding tank which was connected to the seawater system of the lab due to small openings, covered with 50 μm gauze. Thus, organisms maintained in suspension and assured identical feeding requirements for subsequent run. After 1 hour of feeding, the corals tentacle expansion was observed qualitatively with polyps being classified as ‘fully extended’, ‘extended’ or ‘retracted’. Afterwards corals were removed from the chamber. Residual plankton was passed through a 50 μm columnar sieve and preserved in 20 mL of borax buffered 40 % formalin solution. The Kautex bottles were filled up with filtered seawater, closed and

sealed with parafilm before freight to AWI, Bremerhaven. Here, each sample was aggregated in a 50 µm columnar sieve and thoroughly rinsed with deionized water to remove residual formalin solution. The purified plankton sample was then sieved with 1000 µm, 500 µm and 50 µm gauze, respectively, each time rinsed again for 30 seconds to ensure an accurate fractionation. These size-fractionated samples were sorted and counted according to broad taxonomic groups (Newell & Newell 1963).

Capture rates (total prey items cm⁻² projected calyx surface area h⁻¹, % prey items cm⁻² projected calyx surface area h⁻¹) were corrected for internal grazing (0.03 ± 0.01 %). Prey selection was determined taxonomic for each size-class, according Ivlev's (1961) Index E:

$$E = \frac{(r-p)}{(r+p)} \quad \text{Eq. 5}$$

with the frequency of the prey item in the diet (r) and in the environment (p).

2.4.2. Short-term calcification rates

2.4.2.1 Buoyancy Weight Technique

The buoyant weight technique (Jokiel 1978) was used to assess short-term skeletal growth by detecting the increase of accumulated CaCO₃.

Corals were weighed with a Sartorius CPA 225 D balance (± 0.01 mg) directly after termination of the incubation experiments to keep the stress phase for corals as short as possible. They were attached to a screw-nut, which in turn was fixed to the weight-below hook of the balance. Corresponding tank was always identically filled with water of the aquarium system to avoid any errors induced by different water level. Water was cooled by ice packs, ensuring a temperature increase of max. 1 °C during measurements. Prior to each measurement, temperature and salinity of the water was recorded in order to compute the density. As soon as the balance was constant within a range of ± 0.0025

g, corals were weighed 3 times. Values were averaged (wt_{water}) and used for following equation:

$$wt_{air} = \frac{wt_{water}}{\left(1 - \frac{\rho_{water}}{\rho_{CaCO_3}}\right)} \quad (g) \quad \text{Eq. 6}$$

where wt_{air} is the calculated weight in air and ρ_{water} and ρ_{CaCO_3} the seawater and skeletal density, respectively. The skeletal aragonite density (2.835 g cm^{-3}) was taken with reference to Naumann et al. (2011), as it was not possible to supply any coral material for density analyses. Mentioned study used eight *D. dianthus* skeletons and averaged received micro-density results, previously determined according to Davies (1989).

Growth rates (G), expressed in $\% \text{ day}^{-1}$ and mg day^{-1} , are normalized to projected calyx surface area (cm^2) and calculated as follows:

$$G (\% \text{ CaCO}_3 \text{ cm}^{-2} \text{ day}^{-1}) = \left(\frac{(M_{t+1} - M_t)}{(M_t * (T_{t+1} - T_t))} \right) * 100 \quad \text{Eq. 7}$$

$$G (\text{mg CaCO}_3 \text{ cm}^{-2} \text{ day}^{-1}) = \left(\frac{(M_{t+1} - M_t)}{(T_{t+1} - T_t)} \right) * 1000 \quad \text{Eq. 8}$$

with M_t and M_{t+1} as the coral weight in air (mg) at the beginning (T_t) and the end (T_{t+1}) of each growth interval.

2.4.3 Long-term calcification rates

Two different methods were applied in the long-term experiment to assess calcification rates in *D. dianthus*: the buoyant weight technique and the total alkalinity anomaly technique. Calcification measurements were carried out in an interval of 4 weeks.

2.4.3.1 Buoyancy Weight Technique

Buoyancy weight measurements were conducted as described in chapter 2.4.2.1.

2.4.3.2 Total Alkalinity Anomaly Technique

The total alkalinity anomaly technique (Smith & Key 1975) is based on a decrease of two molar equivalents in total alkalinity (TA) for every mol calcium carbonate (CaCO_3) precipitated. Calcification rates were assessed by closed-cell incubation experiments.

Incubation procedure

In a first step corals were carefully brushed without causing any damage of the tissue. Afterwards corals were put separately into SCHOTT glasses (310 mL or 706 mL); the volume chosen according to size of specimens. The 310 mL glasses were equipped with one magnetic stir bar and the 706 mL glasses with two. Incubation glasses were closed airtight under water to enhance the accuracy of the experiments by avoiding equilibration with atmospheric $p\text{CO}_2$. Two controls were run for each size of incubation glass. Corals were incubated for ~ 6 hours with downward facing tentacles. The polyp expansion of each coral was recorded in the middle and at the end of the incubation period as described in chapter 2.4.1. Incubations were always carried out at the same time of the day to avoid any error due to diurnal variations (Edmunds & Davies 1988).

An initial 50 mL TA water sample was taken out of the tank filled with the incubation water. At the end of the incubation period, further TA samples were taken directly out of the control and the coral incubation glasses. All samples were filtrated with a 0.2 μm glass fiber filter (SFCA) attached via a filter holder to a 10 mL syringe (Braun Omnifix®), filled into 50 mL Falcon tubes (Blue Max™), closed gastight and stored at 4 °C pending analysis. The potentiometric titration analysis (Dickson et al. 2007) was conducted

within the following 3 days using a titration unit with associated sample changer (Titroline alpha plus, SI Analytics). 0.05 N hydrochloric acid (Titrisol®) with 32 g of sodium chloride was used to titrate the samples. Samples (25 mL) were measured in duplicates arranged randomly on the automated sample changer. Seawater standards ($n = 5$) were arranged in between, to account for the methodical drift ($\max. \pm 6 \mu\text{mol}$) and were calibrated with Dickson standard Batch 105 (Scripps Institution of Oceanography, San Diego). Prior titration the pH electrode was two-point calibrated using Merck Certipur® buffer pH 4.00 ± 0.01 and pH 7.00 ± 0.01 . Results of a sample were corrected for drift and measurement accuracy, averaged and used for calculation of calcification rates. Calcification rates (G , growth) were calculated according the equation:

$$G = \frac{\left(\frac{\Delta TA}{2}\right) * V_{\text{inc}} * \rho_{\text{SW}}}{A_{\text{calyx}} * t_{\text{inc}}} \quad (\mu\text{mol CaCO}_3 \text{ cm}^{-2} \text{ h}^{-1}) \quad \text{Eq. 9}$$

ΔTA was ascertained by subtracting the control values from those measured in the treatments. The total incubation volume (mL) is represented by V_{inc} . Density of the incubation water (ρ_{SW}) was calculated according to the tables of Bialek (1966) from temperature and salinity measurements, taken of every glass at the end of the incubation. t_{inc} refers to the incubation time (h) of each individual and A_{calyx} (cm^2) was used for normalization of the net calcification rate G .

Jaques & Pilson (1980) have improved the TA method by considering the production or assimilation of inorganic nutrients such as NH_4^+ , NO_3^- and NO_2^- which alters TA.

Therefore, all TA values were corrected according to the formula:

$$\Delta TA = [\Delta TA - \Delta \text{NH}_4^+ + \Delta(\text{NO}_3^- + \text{NO}_2^-)] \quad \text{Eq. 10}$$

The nutrient samples were treated as described in chapter 2.3.3.3.

2.4.4 Long-term respiration rates

Respiration rates were used as indicator for metabolic activity of *D. dianthus* specimens. Pre-experiments at field station *Fundación Huinay* provided preliminary estimates of respiration rates and served as reference for the increase of the incubation period from 4 to 6 hours as the oxygen consumption did not fell below normoxic conditions (80 %) *sensu* Dodds et al. 2007.

The simultaneous effect of flow and food on metabolic activity was examined via a time series of respiration measurements, performed every 4 weeks affiliated to the long-term experiment.

Described incubation experiments (chapter 2.4.3.2) were besides calcification rates also used to study respiration rates. The initial removal of epiphytes was necessary in order to avoid any respiration effects during incubation. Blanks were analyzed to account for background microbial oxygen consumption.

The oxygen consumption was determined at the beginning and the end of each incubation procedure. For pre-experiments, the optode HANNA HI 9828 was used with values documented in a time interval of 5 seconds. The calculated mean ($n = 5$) revealed the remaining oxygen concentration (mg L^{-1}). Previously to each experimental run, the probe was calibrated. The respiration time series was conducted via the oxygen optode HACH LDO_{TM} 101. As soon as logger showed the same oxygen concentration (mg L^{-1}) twice consecutively, probe was stabilized and value was noted.

Total respiration rates (R_T) were derived from the depletion of dissolved oxygen recorded over the incubation period. The calculation was carried out according to the equation below with results being normalized to calyx surface (A_{calyx} , cm^2) of the respective coral:

$$R_T = \frac{\left(\frac{\Delta O_2 * V_{\text{inc}}}{t_{\text{inc}}} \right)}{A_{\text{calyx}}} \quad (\text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}) \quad \text{Eq. 11}$$

ΔO_2 , corrected for background microbial oxygen consumption, represents the difference between oxygen concentration ($\text{mg O}_2 \text{ L}^{-1}$) of treatment and corresponding control. V_{inc}

refers to the incubation volume, calculated by subtracting the displacement volume of the coral (V_{coral} , in mL) from the water volume of a closed incubation glass (V_{glass} , in mL). The total incubation time (t_{inc} , in h) of each coral was calculated as follows: $t_{\text{inc}} = \text{end time of incubation } (t_{\text{out}}) - \text{start time of incubation } (t_{\text{in}})$.

2.4.5 Normalization of physiological data

All data were normalized to projected calyx surface area (cm^2). The planar Projection Photography (Naumann et al. 2009) was chosen for normalization as it appears to be an adequate method for experimental studies that continuously investigate living corals. Therefore, each calyx was photographed (Canon PowerShot® G11/12; resolution 10.0 megapixels) from the top view. Pictures were afterwards processed using the software Image View 1.6. Photography was only carried out if polyps were completely retracted in order to reduce the methodical error. The error of $0.018 \pm 0.027 \text{ cm}^2$ (mean \pm SD) was determined by repeated imaging of one calyx (3x), each again three times digitally encircled to calculate the average calyx area. Calculated calyx surface area (cm^2) was assumed to correlate with polyp diameter and was therefore used as coral size reference.

2.5 Statistical analyses

Statistics were performed with different tests provided by the software SigmaPlot[®] Version 11.0.

Neither evaluated short-term growth rates of *D. dianthus* nor the raw data of percentage capture rates fulfilled the demands of normal distribution. Thus, a non-parametric two-sided Mann-Whitney-U test was applied to check for significant differences between treatments.

In contrast, all remaining data sets used for analyses passed normality and equal variance tests. The One-way analyses of variance (ANOVA) compared the different treatments and tested for an overall significance. In case of significance, further utilized multi-comparison tests analyzed all possible pairs of means to get the responsible matches. Accordingly, Tukey's range test was chosen for comparisons of metabolic activity amongst treatments whereas the more powerful Holm-Sidak test was applied for the comparative evaluation of calcification rates. The Two-way analyses of variance was run for both, metabolic activity and calcification of corals and assessed if findings were attributable to investigated parameters (flow and food status) or if they were caused by random variations. Correlation of metabolic activity and calcification was determined via linear regression analyses.

3 Results

3.1 Feeding Experiment

Feeding experiments were carried out to examine flow depended capture rates of *D. dianthus*. Results are given in either total rates (Fig. 3.1 A) or percentage rates separated for the different prey size classes (Fig 3.1 B).

Strongest experimental flow speed (4.3 cm s^{-1}) depressed the capture success, illustrated in figure 3.1 A by lowest values of $25 \pm 5 \text{ prey items cm}^{-2} \text{ h}^{-1}$ (mean \pm SEM). At low flow speeds of 1.7 cm s^{-1} capture rates were as twice as high with values of $55 \pm 16 \text{ prey items cm}^{-2} \text{ h}^{-1}$ (mean \pm SEM). The largest variability of replicates was evaluated under stagnant conditions (0 cm s^{-1}) with a total capture rate of $31 \pm 19 \text{ prey items cm}^{-2} \text{ h}^{-1}$ (mean \pm SEM). However, the statistical analyses (Mann-Whitney U test, see Appendix Table A1) revealed no significant differences in capture rates within the three flow regimes.

A comparison of prey size fractions amongst treatments (Fig. 3.1 B) showed no significant difference with flow (Appendix, Table A2). Further comparative evaluation of prey size fractions within each flow treatment were used to determine a size dependent food uptake. Even though the $> 1000 \text{ }\mu\text{m}$ size fraction represents $\sim 50 \%$ of captured food, none of the treatments revealed statistically significant differences (Appendix, Table A3). However, largest shift in composition can be assumed at the highest flow speed (4.3 cm s^{-1}) with an increase of $\sim 10 \%$ of the mid-level size class ($500 - 1000 \text{ }\mu\text{m}$).

Also selectivity on species level was tested within each size-class (according to Ivlev, see Appendix, Table A4). Results revealed euphausiids ($> 1000 \text{ }\mu\text{m}$), cladocerans ($500 - 1000 \text{ }\mu\text{m}$), copepoda nauplii ($500 - 1000 \text{ }\mu\text{m}$) and ostracods ($< 500 \text{ }\mu\text{m}$) as the only positive caught organism at all flow speeds within respective size-fraction. Photographs of examined zooplankton organisms are supplementary illustrated (Appendix 8).

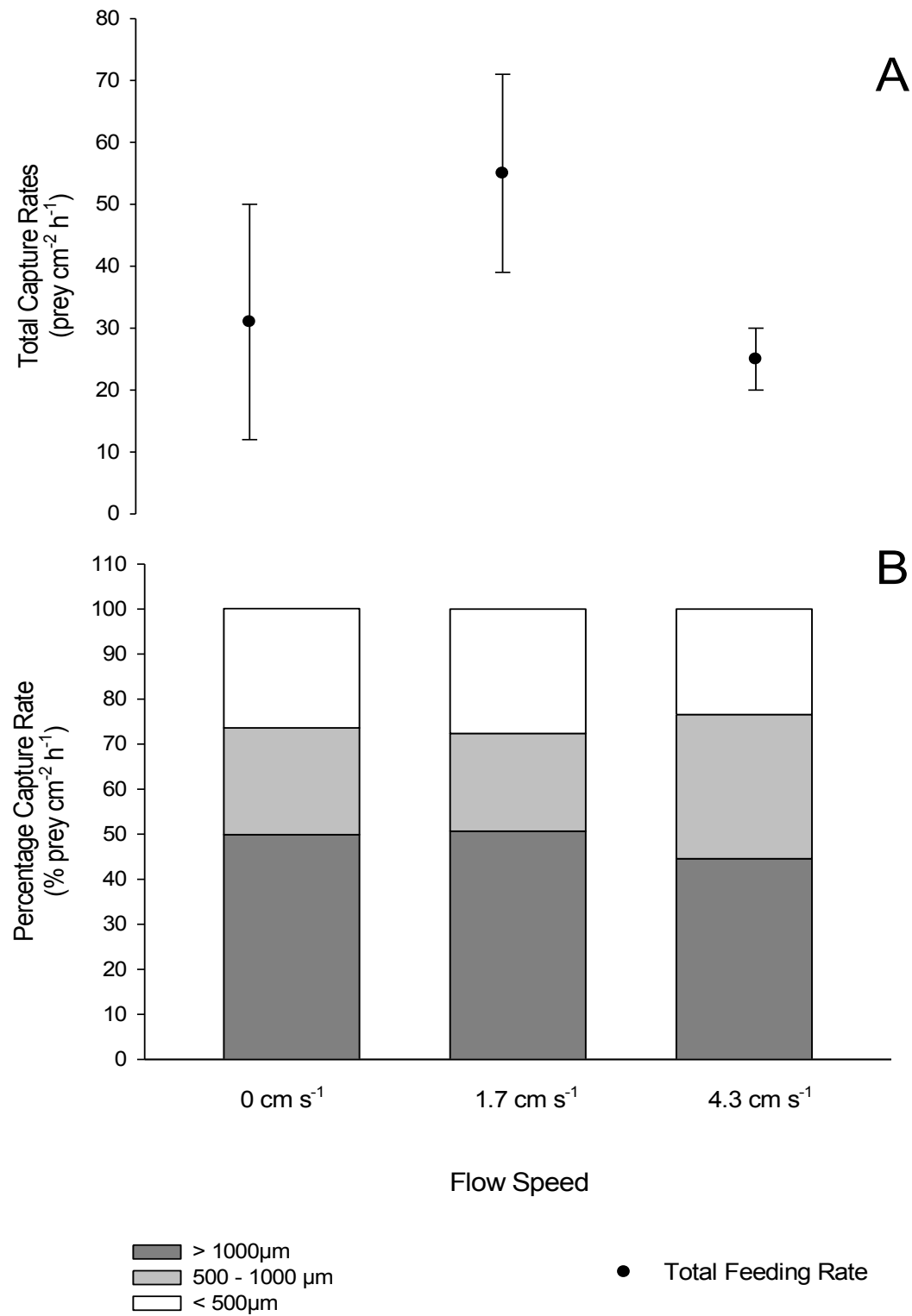


Fig. 3.1: Capture rates of *D. dianthus* referred to three different flow speeds (0cm s⁻¹, 2cm s⁻¹, 5cm s⁻¹) in (A) total prey (median ± standard error) cm⁻² h⁻¹ and (B) proportional distribution of prey size classes (> 1000 μm, 500 – 1000 μm, < 500 μm) within captured food (% prey cm⁻² h⁻¹).

3.2 Short-term calcification rates

Twenty specimen of *D. dianthus* were simultaneously maintained for 14 - 16 days *in situ* and in the lab, respectively (*in situ*: n = 19 after Outlier test; lab: n = 16 after Outlier test). Corals grown *in situ* reveal calcification rates with mean values (\pm SD) of 5.44 ± 3.45 mg $\text{CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$ (min = 0.87, max = 14.48) and a mass increase of 0.25 ± 0.18 % d^{-1} . Calcification rates of specimens maintained in the lab are about one-third lower. Precipitated calcium carbonate displays a mean (\pm SD) of 1.86 ± 1.37 mg $\text{CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$; (min = 0.0009, max = 5.44) and mass increase of 0.09 ± 0.08 % d^{-1} . Short-term calcification rates (Fig. 3.2) show highly significant differences ($p = < 0.001$, Mann-Whitney-U test) between corals grown *in situ* and in the lab.

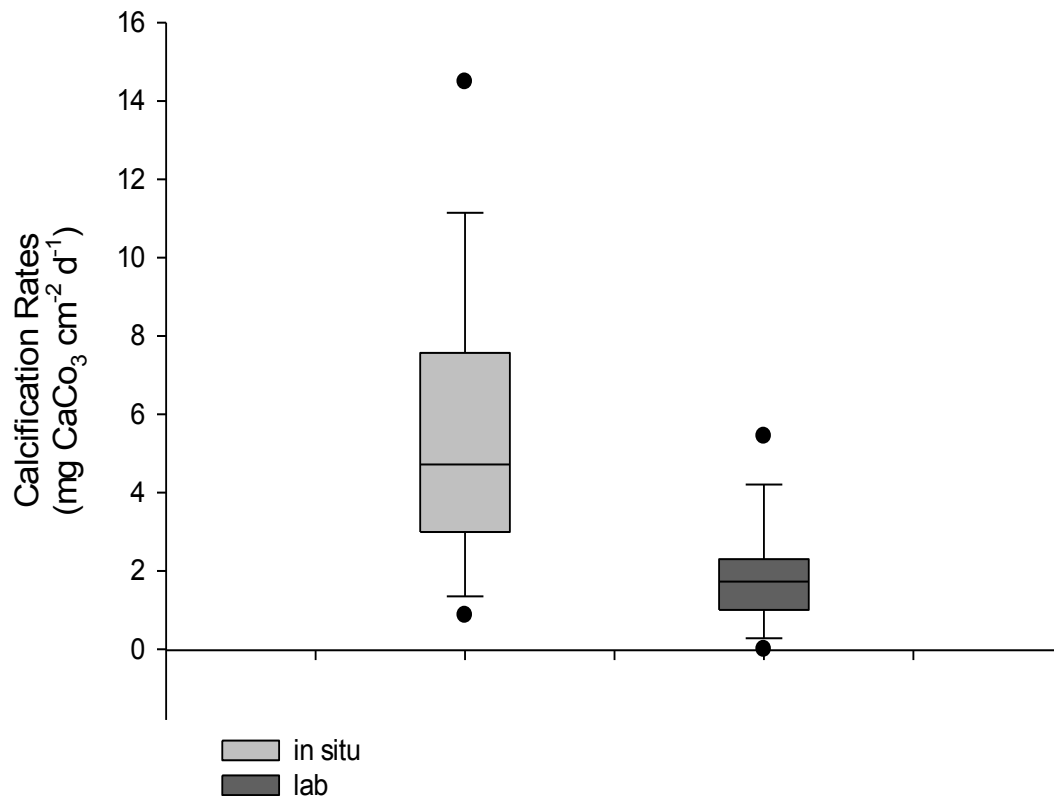


Fig. 3.2: Short-term calcification rates of *D. dianthus* given in mg $\text{CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$, comparative illustrated for individuals maintained for 14-16 days *in situ* (light-grey boxes) or in the lab (dark-grey boxes). Box-Whisker-Plots represent the interquartile range of replicates (colored part) with median (black horizontal line), standard deviation (whiskers) and min/max values (black dots).

3.3 Long-term calcification rates

This chapter covers the results of long-term calcification rates of *D. dianthus* maintained with exposure to different current strength and food concentrations (see chapter 2.3.3 for exact values). The four treatments are termed in the following as LFLC (low food + low current), LFHC (low food + high current), HFLC (high food + low current) and HFHC (high food + high current).

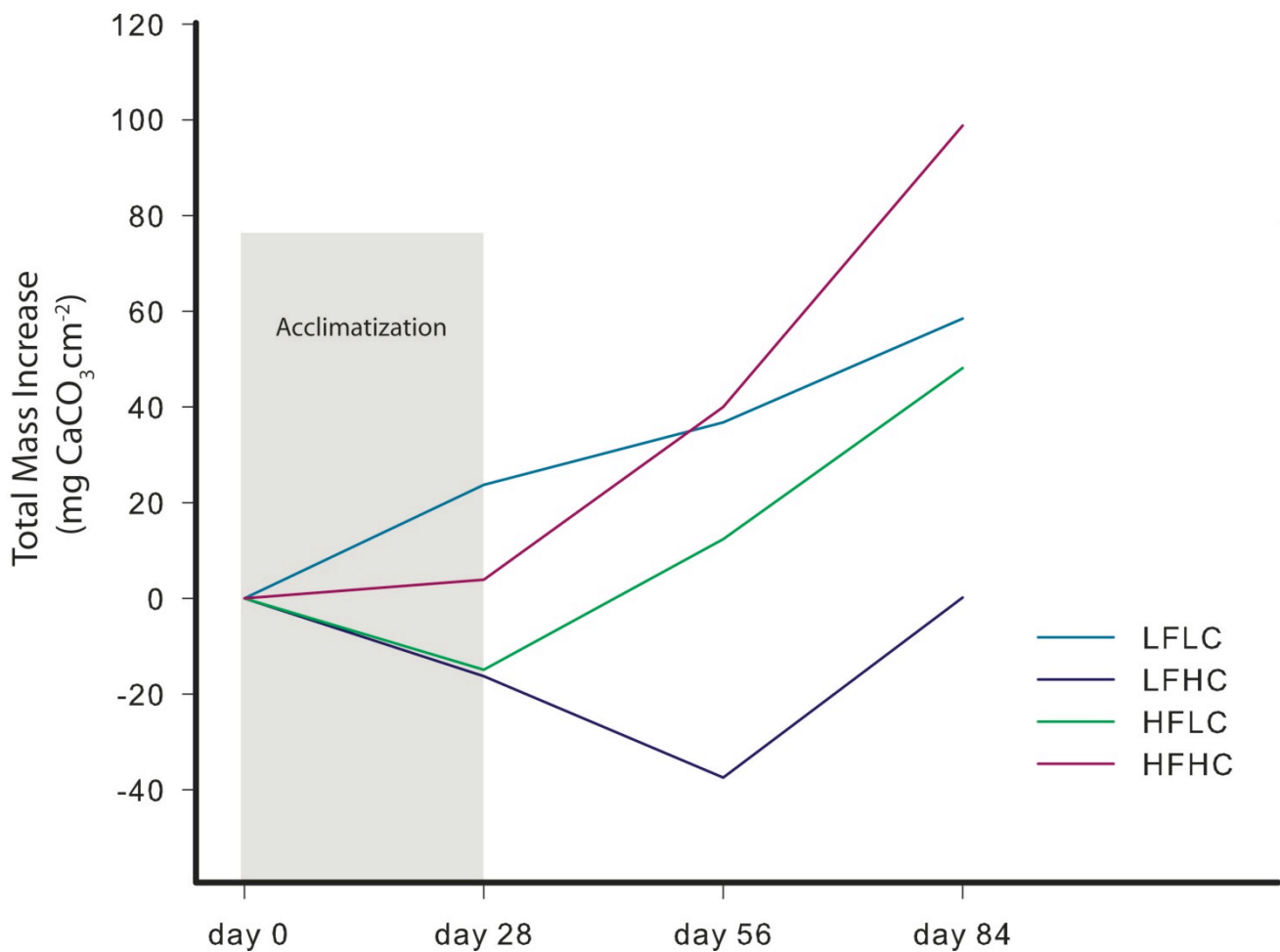


Fig 3.3: Total mass increase in mg CaCO₃ cm⁻² against time (days) for each treatment (replicates: n=9) of the long-term experiment. Treatments differ in food concentration (F) and current strength (C) both adjusted to low (L) and high (H) conditions. First time interval (day 0 to day 28) reveals the acclimatization period.

The averaged total mass ($\text{mg CaCO}_3 \text{ cm}^{-2}$) increase of corals over the entire experimental time span is diagrammed in figure 3.3. Treatments, each consisting of nine replicates, show complete different growth curves. During the first 28 days of cultivation (acclimatization period) a sharp decline of two treatments (LFHC, HFLC) can be noticed. A general positive trend can be observed throughout the last two time intervals (day 28 to day 84) except for treatment LFHC.

Using day 28 as initial point (t_0) for mass increase determination, HFHC displays highest growth records ($94.1 \pm 46.6 \text{ mg CaCO}_3 \text{ cm}^{-2}$). Although the second treatment under fed conditions (HFLC) reduced weight while acclimatization, corals regenerated and gained $63.0 \pm 45.0 \text{ mg CaCO}_3 \text{ cm}^{-2}$. These fed treatments reveal a similar growth pattern with the steepest slope belonging to the last time interval, indicating a successive rise in growth speed over time.

The curves of the low food treatments represent the smallest amounts of calcium carbonate accumulation. A consistent but in general slight rising slope can be observed in corals within the LFLC treatment. It reaches with an overall mass increase of $34.7 \pm 23.1 \text{ mg CaCO}_3 \text{ cm}^{-2}$ approximately 50 % less than treatment HFLC. Inclusion of the acclimatization period (Fig. 3.3) causes a conversely effect with a depressed HFLC treatment, hence, merely attributable to its decreasing trend within the first 28 days. Lowest total mass increase ($16.4 \pm 23.1 \text{ mg CaCO}_3 \text{ cm}^{-2}$) was found in treatment LFHC which can be clearly referred to the weight loss computed up to day 56. This period of decline followed an immense increase in mass, even comparable as found in treatment HFLC.

The calcification rates ($\text{mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$) of treatments vary significantly ($p < 0.002$, One Way ANOVA), displayed below in figure 3.4. Growth proceeded within acclimatization period was neglected for calculations.

Lowest calcification rates were visible in treatments where corals starved (Table 5). A percentage mass increase of $0.03 \pm 0.03 \% \text{ d}^{-1}$ and calcification rate of $0.63 \pm 0.42 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$ could be detected under low current conditions (LFLC), whereas starving in combination with high flow speeds (LFHC) appears to intensify the reduction of calcification rates ($0.3 \pm 0.58 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$; $0.01 \pm 0.02 \% \text{ mass increase d}^{-1}$). Nevertheless, the Holm-Sidak Test proved no significant difference between those two treatments (see report in Appendix 4).

In contrast, fed treatments with general higher calcification rates show an increase with rising flow though it failed to be significant. Calcification rates of treatment HFLC were in average $1.1 \pm 0.8 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$, with a mass increase of $0.05 \pm 0.03 \% \text{ d}^{-1}$. Highest growth rates of the experiment occur under high food concentrations combined with strong flow speeds (HFHC). Here, calcification rates show values of $1.7 \pm 0.9 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$ and a mass increase of $0.07 \pm 0.05 \% \text{ d}^{-1}$.

Significant differences ($p < 0.001$, $p < 0.004$) are visible by comparing HFHC with the unfed treatments LFHC and LFLC, respectively. The Two Way Analyses of Variance failed to be significant. Therefore, it could not be excluded that the differences in mean values are just due to random sampling variability.

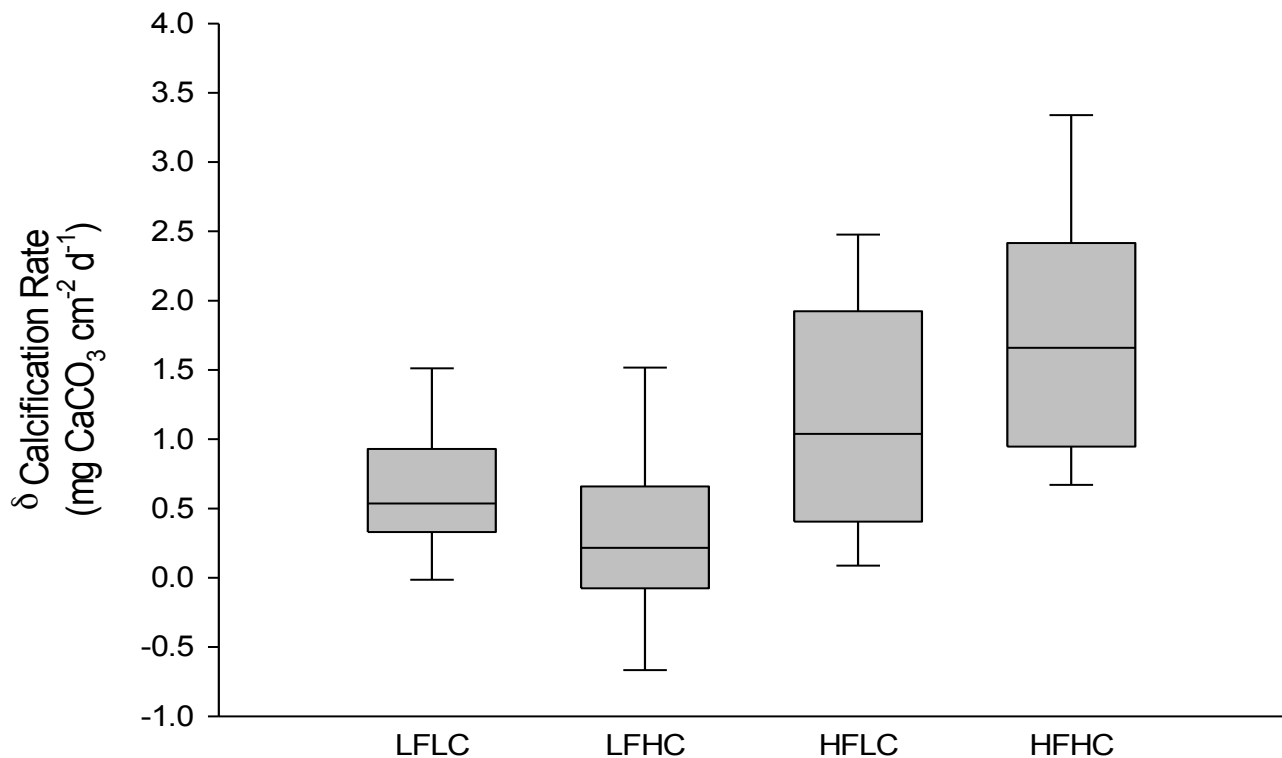


Fig. 3.4: Averaged calcification rates ($\text{mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$) of treatments, differing in food concentration (F) and current strength (C) both adjusted to low (L) and high (H) conditions. Plotted calcification rates were evaluated over a period of 56 days (δ value) and reveal the median of each treatment (black horizontal line), the interquartile range containing 50 % of spread values (grey box) and the standard deviation within replicates.

3.3 Long-term changes in metabolic activity

Figure 3.5 displays the shift of respiration rates ($\text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) during the long-term experiment, evaluated for the time interval between day 28 and day 84. Results are used as an indicator for changes in metabolic activity.

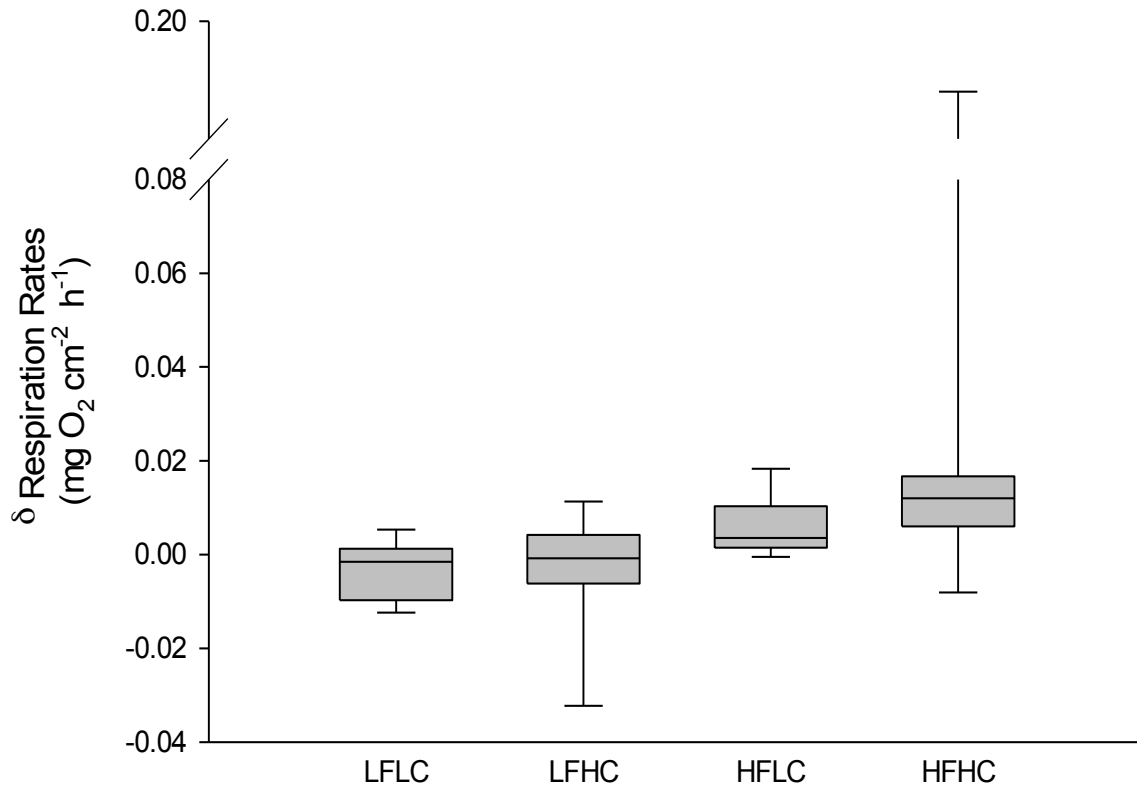


Fig. 3.5: Respiration rates ($\text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) as indicator for metabolic activity, here evaluated over a period of 56 days (δ value). The four treatments differ in food concentration (F) and current strength (C) which in turn are adjusted to low (L) and high (H) conditions. Slash marks interrupt the y-axis from 0.08 to 0.18 $\text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$, the whisker of box-plot HFHC is interrupted within the same range.

The means of the four treatments show a significantly increasing trend ($p < 0.002$, Appendix 6) from low food and low current strength conditions (LFLC) to enhanced conditions (HFHC). Although the midspreads of all treatments are located within a narrow range, the HC treatments reveal a high variability between replicates which in turn rise with increasing food concentration ($\text{LFHC} = -0.003 \pm 0.012 \text{ mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$,

HFHC = $0.028 \pm 0.053 \text{ mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$). Moreover, corals of the LFHC treatment show the strongest reduction of metabolic activity, whereas the greatest increase is found from individuals placed within HFHC (Table 6). The Tukey Test proved the HFHC treatment significant different ($p = < 0.05$) compared to the low food treatments (LFLC = $-0.004 \pm 0.006 \text{ mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$, LFHC = $0.006 \pm 0.006 \text{ mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$). Nevertheless, differences cannot be referred to levels of food or flow, but rather are expected to be caused by random variations.

Table 6: Summarized results of respiration rates ($\text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) and calcification rates ($\text{mg CaCO}_3 \text{ cm}^{-2} \text{ h}^{-1}$). Treatments differ in food concentration (F) and current strength (C), each adjusted to either low (L) or high (H) conditions. Given values are the mean rates ($n = 9$), the Standard Deviation (SD), the Standard Error (SEM) and the minimum and maximum rates of each treatment.

Treatment	Respiration Rate ($\text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$)		Calcification Rate ($\text{mg CaCO}_3 \text{ cm}^{-2} \text{ h}^{-1}$)	
LFLC	-0.004	SD: 0.006 SEM: 0.002 min: -0.0094 max: 0.0053	0.63	SD: 0.42 SEM: 0.14 min: -0.01 max: 1.51
LFHC	-0.003	SD: 0.012 SEM: 0.004 min: -0.032 max: 0.011	0.30	SD: 0.58 SEM: 0.20 min: -0.66 max: 1.5
HFLC	0.006	SD: 0.006 SEM: 0.002 min: -0.005 max: 0.018	1.7	SD: 0.85 SEM: 0.28 min: 0.6 max: 2.48
HFHC	0.028	SD: 0.053 SEM: 0.018 min: 0.008 max: 0.176	1.15	SD: 0.82 SEM: 0.27 min: 0.67 max: 3.3

3.4 Relationship between metabolic activity and mass increase

The relationship of metabolic activity ($\text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) on the corals mass increase ($\text{mg CaCO}_3 \text{ cm}^{-2}$) is represented by a linear regression model (Fig. 3.6). Used values base on the last time interval of the long-term experiment (day 57 - 84).

The linear trend indicates a positive correlation with a significant relationship ($p < 0.001$, Appendix 7) between respiration and mass increase. Thirty four percent of the values lie within the confidence band, another 63 % within the prediction band. One data point is located beyond these ranges. Another outlier was previously eliminated due to a test which implements the criterion of multiple standard deviations.

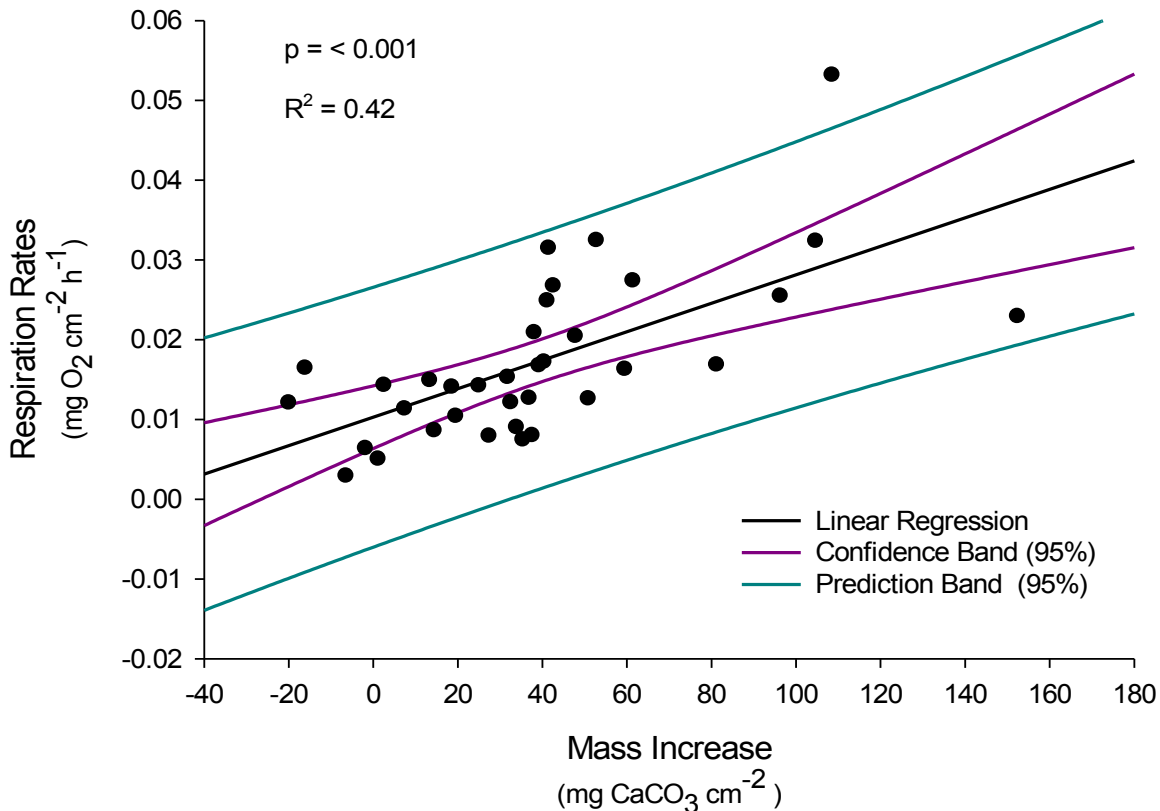


Fig. 3.6: Relationship between respiration rates ($\text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) and mass increase ($\text{mg CaCO}_3 \text{ cm}^{-2}$). Values base on the last time interval of the long-term experiment (day 57 to 84) and comprise all treatments (36 corals, due to outlier test here only 35 corals). The black line reveals the linear correlation of respiration rates and mass increase. The confidence band (95 %) is indicated by the purple lines, the prediction band (95 %) by the blue lines. Significance level (p) and coefficient of determination (R^2) refer to the linear regression analysis.

4 Discussion

4.1 Feeding of *Desmophyllum dianthus*

Little is known about the diet of cold-water corals and also feeding rates were rarely examined so far. To the author's best knowledge, this was the first study investigating capture rates of *D. dianthus* under varied flow conditions and its preference to prey size classes and therein located zooplankton species. It should be noted that the following conclusions rely on tendencies, as the feeding results could not be proved to be significant. Nevertheless, an overall conclusive pattern could be observed and is discussed in the following.

The efficiency of capture rates seems to decrease with increasing flow velocity. Highest total capture rates were observed under moderate flow speeds of 1.7 cm s^{-1} whereas already an increase in velocity to 4.3 cm s^{-1} capture rates by half depressed (Fig. 3.1 A). This flow depended capture success has been already described for barnacles (Trager et al. 1994), octocorallia (Sebens 1984) and also for the branching cold-water coral *Lophelia pertusa* (Purser et al. 2010). The latter study exposed *L. pertusa* to different concentrations of *Artemia salina* nauplii and varying flow speeds (2.5 and 5.0 cm s^{-1}), comparable to the present study. Again, they found maximum net capture rates at lower flow speeds although the delivery of food particles is known to be enhanced by increasing flow velocities. This contradiction can be explained by the tentacle deformation of the polyp. Personal observations and former studies on soft corals (Fabricius et al. 1995), gorgonians (Dan & Lin 1993) and tropical scleractinians (Sebens & Johnson 1991) report a progressively greater bending of tentacles with raising flow. This drag effect reduces the ability for actively controlled movements which in turn results in less successful prey capture and ingestion rates (Patterson 1991a, Eckman et al. 1993).

Corals incubated under stagnant conditions revealed a capture rate of 31 ± 19 prey items $\text{cm}^{-2} \text{ h}^{-1}$. The distinct increase of capture rates from stagnant to moderate flow conditions can be referred to enhanced encounter rates favorable for passive filter feeders. In general, determined capture rates reveal a clear optimum curve with flow. This pattern of depressed capture rates to both sites of a narrow range of flow speed

which appeared to be most beneficial has been demonstrated also for alcyonacean corals (Mc Fadden 1986) and gorgonian corals (Sponaugle & LaBarbera 1991).

By now, only one study investigated prey-capture rates of *D. dianthus* (Tsounis et al. 2010). They exposed specimens to either *A. salina* nauplii or *A. salina* adults and obtained capture rates of 93.23 ± 47.65 and 8.48 ± 2.97 Ind. Polyp⁻¹ h⁻¹ (\pm SD), respectively. Taking into account that their incubations were conducted at flow speeds of 1 cm s⁻¹, the results are only comparable with the rates at stagnant or moderate flow conditions (1.7 cm s⁻¹) of this study. Furthermore, it has to be kept in mind that capture rates of the present study were normalized to square centimeter projected calyx surface area. Accordingly, Tsounis et al. (2010) detected lower capture rates which can be explained by several experimental differences. They added 23.53 *A. salina* L⁻¹ in a 850 mL incubation chamber and terminated the experiments when prey quantity remained constant (30 min – 2 h). The present study supplied a natural collection of zooplankton in higher concentrations (*ad libitum*). This is referred to Palardy et al. (2006), who found a positive correlation between plankton concentration and capture rate, a considerable reason for higher capture rates even if corals were incubated in 3 L jars. Hence, absolute values of these studies have to be interpreted with caution since they may be strongly influenced by the corresponding experimental design. Nevertheless, capture rates of the present study might be even underestimated as the amount of deposited plankton during experiments was not quantified. Due to the freshness of caught plankton and their storage in tanks connected to the flow-through system of the lab, decrease during incubations was expected to be low. This assumption was strengthened by observant monitoring during incubations.

Furthermore, Tsounis et al. (2010) suggested *A. salina* nauplii (250 – 500 μ m) within the optimal prey size range, contrary to the findings of this study. Feeding experiments showed that roughly 50 % of captured food was covered by prey items above 1000 μ m (Fig. 3.1 B). This can be attributed to the morphology of *D. dianthus*, specialized due to large polyps and wide-set tentacles. An enlarged tentacle crown diameter may also result in greater nematocyst adhesion capabilities, an advantage for capture of large prey items. Findings are furthermore in accordance with Sebens et al. (1996) who found polyp size positively correlated with captured prey size. Chamber artifacts may be an additional explanation. Larger prey might be more often harmed by the corals nematocysts than smaller, triggered by the water volume to prey size ratio. Although

large prey is initially able to disentangle in some cases, it results in a successive distinction and increasing chance to get finally caught. Another critical aspect which has to be considered is the general escape behavior of certain organisms. Copepods, the dominant taxon found within the 500-1000 μm size fraction, are capable to perform fast escape responses to avoid predation. This ability decreases with increasing flow (Robinson et al. 2007), also indicated by the results of the present study. The highest captured amount of the 500 - 1000 μm size fraction was found under the strongest experimental flow speed (Fig. 3.1 B).

Utilized prey selection indices revealed a more obvious pattern. Relatively large individuals (e.g. euphausiids) and individuals with weak swimming capabilities (e.g. nauplii) were captured to a greater extent than strong swimming copepods, even when highly abundant. Prey selection indices applied on passive suspension feeders can be, thus, only used as indirect measure for prey escape ability instead for predation preference. This was also concluded by Sebens et al. (1998).

In summary, results indicate that differences in capture rates of prey-size classes are related to the anatomy of *D. dianthus* and the escape behavior of potential prey rather than to any selectivity by the coral.

Nevertheless, findings can be used for comparative analyses between treatments but only restricted for assessment of natural capture rates. Transferring laboratory results to *in situ* conditions should be always done with caution due to complete different environmental conditions. However, capture rates of the present study are assumed to be more representative than those from Tsounis et al. (2010), primarily due to the supply of the coral's natural food spectrum under consideration of their endogenous circadian rhythm. Feeding experiments were carried out each night consistently between 21:30 and 1:00 (local time) when activity and corresponding tentacle expansion of *D. dianthus* was significantly increased (Maier 2010, unpublished data) presumably to feed on diurnal migrating zooplankton (Heidelberg et al. 2004).

The high variations of replicates (Fig. 3.1) which in turn influenced the statistical analyses can therefore not be derived from temporal differences in activity patterns. Monitored data illustrated that corals were always 'fully extended' during experiments. Variations can be rather attributed to the naturally changing zooplankton

concentrations on each experimental day, for some extent, but assuredly to inter-individual differences in coral size and behavior.

4.2 Metabolic Activity

This study used changes in respiration rates as an indicator for varying metabolic activity and corresponding coral fitness. Aim was, to evaluate the cross-effects of feeding and flow on the coral's metabolic activity and therewith the environmental importance of these two parameters.

A comparison of the four treatments demonstrated an increasing trend in respiration with enhanced flow conditions and food availability (Fig. 3.5).

Profound effects of water motion on coral ecology have been described in several former studies: for their distribution (Vosburgh 1977), their recruitment (Sammarco & Andrews 1988) and for their metabolism in general (Patterson et al. 1991b, Shashar et al. 1993, Finelli et al. 2006). Corals lack the ability to actively ventilate their external surface, they are known as oxyconformers (Patterson 1992). This leads to a strong dependency on current strength to enhance gas exchange with the adjacent water column. The exchange rates are in turn strongly influenced by the thickness of the diffusive boundary layer (DBL) surrounding each coral. Increasing flow speeds cause a thinning of the DBL and leads to reduction of the distance that molecules must traverse via Brownian motion (Shashar et al. 1996). That flow significantly increases respiration rates was also proven by pre-experiments of the present study. Same corals were kept for four hours on two consecutive days in either stirred or unstirred glass-beakers, which resulted in significantly varying respiration rates. Nonetheless, described experiment comparative evaluated stagnant conditions to conditions with water motion. By comparing respiration rates of the long-term experiment where corals were maintained under flow speeds of $\sim 2 \text{ cm s}^{-1}$ and $\sim 5 \text{ cm s}^{-1}$, no significant differences could be detected. This relies most likely on the consistent high oxygen saturation (103.4 %) of tank water during the long-term experiment with high concentration gradients favoring the passive diffusion through the DBL (Finelli et al. 2006). The

relatively small range in flow speed, at least compared to natural flow fluctuations, could furthermore masked possible effects. Also the unusual and varying occurrence of endolithic greenalga and cyanobacteria is conceivable to alter respiration rates independently from flow velocity. The endoliths are located beneath the tissue and thereby within the microhabitat created by the DBL. In case of photosynthetic activity, they may provide oxygen for the coral's metabolism and may therefore reduce the importance of flow for oxygen supply. Another potential source of error could be chamber artifacts. Although the corals were arranged in a staggered manner to avoid variations in flow exposure (e.g. turbulences, flow speed...), there may be differences as flow speed was not completely homogenous throughout a chamber (Table 4).

However, the only significant differences within the long-term experiment were achieved by comparison of the two food treatments. This may indicate food availability of greater importance for general coral fitness than flow conditions.

Grotolli et al. (2006) already showed a positive correlation between enhanced food ingestion and bleaching resilience of zooxanthellate warm-water corals. The high relevance of zooplankton availability for the respiratory metabolism of *D. dianthus* is shown by the study of Naumann et al. (2011). They obtained a progressive decrease of respiration rates with end of zooplankton supply and explained that with regard to the metabolic budget of *D. dianthus*. Ingested zooplankton ($46 \pm 5 \mu\text{mol POC g}^{-1} \text{ d}^{-1}$) under fed conditions represented approximately the 1.6-fold of the coral's daily respiratory C demand. Pre-experiments of the present study ascertained a respiratory carbon loss of $9.78 \pm 3.88 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ for growing *D. dianthus* specimens. However, due to the different units though difficult to compare. Still, the results of Naumann et al. (2011) may be comparable to the fed treatment of the long-term experiments, since corals were maintained under similar experimental conditions (food supply, current speed, temperature).

In conclusion, the present study provides evidence that heterotrophy functions as a major organic C source for respiratory processes, hence, positively affects metabolic activity. However, it cannot confirm the assumption of Naumann et al. (2011) that metabolic respiration could get fully sustained by it.

A combination of strong flow speeds and high food concentrations (when prey directly supplied) seem to be the most optimal conditions for *D. dianthus* specimen.

Corals maintained under these conditions revealed the highest metabolic activity, but also the greatest variation in replicates (Fig. 3.5). This can partly be explained by inter-individual differences which may appear more intense under favorable environmental conditions, e.g. the lack of external stressors.

Strongest decrease of coral fitness was obtained under starvation and high flow speeds – emphasizing food concentration again as a major parameter influencing the metabolic activity of corals. An explanation can be found by consulting the outcomes of the feeding experiments. Thereafter, *D. dianthus* appears to capture most at moderate flow speeds of 1.7 cm s^{-1} with a strong decreasing trend towards higher flow speeds of 4.3 cm s^{-1} . These findings are relatable to the long-term experiment due to comparably adjusted flow velocities. The water used for regular water exchange was unfiltered North Sea water, hence, still containing microzooplankton and to a lesser extent mesozooplankton organisms. Even if organisms were relatively fast removed from the water column via the filter system, this might have been an additional food source for starving corals within the low flow treatments.

Nonetheless, applied Two Way ANOVA proved the significantly different respiration rates of *D. dianthus* to rely on sample randomness rather than on treatment effects. This depends most likely on the relatively low replicate number available for investigations and is merely related to the experimental approach and not to *in situ* conditions.

Conditions experienced by corals in the experimental chambers differ to those in the field most notably in flow dynamics. The chamber set-up produces a laminar and unidirectional flow regime whereas corals *in situ* experienced rather turbulent and tide dependent bidirectional current regimes (Finelli et al. 2006). Turbulent flow is commonly known to generate the formation of small eddies at the DBL. The concluding shift from molecular diffusion to the more rapid eddy diffusion enhances the exchange rates between coral tissue and surrounding water column with potentially beneficial consequences (Gardella & Edmunds 2001). Accordingly, respiration rates of *D. dianthus* might be even higher under natural conditions as recorded in experimental approaches.

Overall, corals had acclimatized to their treatments. Their respiration rates and corresponding changes in metabolic activity were measured every 4 weeks in dedicated incubation chambers – all under same flow conditions. Even though flow velocity within the incubation chambers had influenced the temporal oxygen consumption to a certain degree, treatment related respiration rates revealed significant differences.

It has to be noted that temperature variations of ± 0.5 °C were measured between incubation chambers to end of the runs. The study of Dodds et al. (2007) showed an increasing oxygen consumption of about 50 % when temperature was raised by 2 °C. Nevertheless, this was demonstrated for deep-dwelling *L. pertusa* specimens naturally exposed to relatively stable temperature conditions. *D. dianthus* specimens used for the present study were collected at 20 m water depth, means strongly influenced by vertical mixing processes within the fjord and, thus, presumably less sensitive to temperature changes. Furthermore, it has to be considered that respiration rates were measured between 9:00 and 15:00 pm, the daytime when *D. dianthus* commonly retract its tentacles (Maier 2010, unpublished data). Monitored activity patterns during respiration measurements varied markedly and showed a positive correlation between tentacle extension and oxygen consumption, presumably due to an increased surface area to volume ratio (Levy et al. 2001). These differences in activity are a possible explanation for the spread of replicates and could have been scaled-down if the endogenous circadian rhythm of *D. dianthus* would have been minded. A reduction of respiratory processes via oxygen depletion within incubation chambers can be excluded as potential source of error, even for treatments with high rates of metabolic activity. None of the oxygen levels fell below 8.2 mg L⁻¹, which is still the upper range of oxygen concentration recorded for the surface layer of fjord Comau (Silva 2008).

4.3 Relationship between metabolic activity and calcification

Metabolic respiration and calcification rates of *D. dianthus* specimens were positive correlated (Fig. 3.6). This implies that these two processes might be related. Possible reasons, including molecular mechanisms, will be discussed in the following.

The main functions of metabolic respiration are (1) the production of ATP to support energy-requiring physiological processes and (2) the release of respiratory CO₂ (Willmer et al. 2005), both considered to effect coral calcification.

Generally, calcification can be described as an energy-consumptive process. Despite the synthesis of organic molecules (0.1 -1 % of skeleton), also the poorly understood pathways of ion transport and carriage demand large amounts of energy (Allemand 1998). Investigations of Barnes & Chalker (1990) showed that corals maintain a very low intracellular Ca²⁺ level compared to the surrounding seawater which needs highly productive and therefore energy-consumptive pumps. The Ca²⁺/H⁺-ATPase, located at the calciblastic epithelium, pumps Ca²⁺ against its concentration gradient into the calcifying fluid under coincidental exchange of protons (Tambutté et al. 1996). This removal of protons results in an increase in pH and aragonite saturation within the calcifying fluid which in turn leads to precipitation of aragonitic calcium carbonate (Al-Horani et al. 2003). Taking into account that former studies report numerous mitochondria in the calciblastic epithelium of corals (Johnston 1980), it is assumable that energy required for the skeletogenesis driving Ca²⁺/H⁺-ATPase is directly derived from respiratory-produced ATP.

These large amounts of mitochondria indicate furthermore that metabolically produced CO₂ might be an additional important source for coral calcification. Although the chemical origin of DIC used for calcium carbonate accretion is still under discussion (Allemand et al. 2011), incorporation of respired CO₂ has been previously reported for tropical scleractinia (Furla et al. 2000) and non-symbiotic gorgonians (Allemand & Grillo, 1992). Further studies of Adkins et al. (2003) and Naumann et al. (2011) verified it also for the cold-water coral *D. dianthus*.

Adkins et al. (2003) determined light stable isotopes, or to be more precise $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, within the skeleton of *D. dianthus* and used it as a powerful tracer to detect metabolically derived carbon. Even if the signature was stronger in the cold-water coral *L. pertusa*, they found significant amounts of incorporated respired carbon.

Naumann et al. (2011) computed a metabolic budget for *D. dianthus* which suggests, contrary to the findings of Adkins et al. (2003), respiration as a predominant carbon source for calcification. Seventy percent ($\sim 27 \pm 10 \mu\text{mol C g}^{-1} \text{ day}^{-1}$) of inorganic carbon implemented by calcification was assumed to stem from respiratory processes.

Based on those considerations and with regard to the findings of the present study, it can be concluded that respiratory processes do influence calcification rates in the cold-water coral *D. dianthus*. Nevertheless, information on physiology and existing ion carriers of *D. dianthus* is too little to figure out how these two processes might interact.

4.4 Calcification of *Desmophyllum dianthus*

4.4.1 Short-term calcification rates

For the first time short-term calcification rates of *D. dianthus* were compared between specimens grown *in situ* and in the lab. The significantly higher *in situ* calcification rates (Fig. 3.2) provide evidence that growth rates obtained in laboratory setups cannot be used as reference for their natural growth behavior.

In general, cold-water corals are known to be considerably less productive than their warm-water relatives (Freiwald et al. 2004). Highest cold-water coral growth rates were reported by Gass & Roberts (2006) for *L. pertusa* colonies ($26 \pm 5 \text{ mm yr}^{-1}$), grown *in situ* on oil gas platforms in the North Sea. By contrast, up to 150 mm yr^{-1} is noted for tropical scleractinian colonies (Spalding et al. 2001). These lower growth rates of cold-water corals are caused by the lack of energy-delivering photoautotrophic symbionts and by the exposure to cold water masses down-controlling their metabolic activity (van't Hoff Q_{10} rule). Nonetheless, measured growth rates of shallow living Chilean *D. dianthus* specimens are in the same range as those of massive and slow growing warm-water *Porites* species (Bak 1990). Findings are in agreement with the study of Orejas et al. (2011b), demonstrating comparable growth rates between the cold-water coral *Madrepora oculata* and the massive tropical coral *Galaxea fascicularis*. These results confirm that cold-water corals can grow at similar rates to those of some slow growing tropical species.

Detected *in situ* growth rates of *D. dianthus* ($5.44 \pm 3.45 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$; $0.25 \pm 0.18 \%$ mass increase d^{-1}) may be even underestimated concerning the extensive handling

procedure (collecting, preparing, weighing, bringing out, re-collecting, re-weighing) which specimen experienced in a relatively short time span of 2 weeks. However, coincident maintained corals in a flow-through aquarium system reached only about one-third ($1.86 \pm 1.37 \text{ mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$; $0.09 \pm 0.08 \text{ \% mass increase d}^{-1}$) of growth rates detected *in situ*.

This can be referred to manifold reasons but most probably to differences in food availability. As shown above (chapter 3.3, 3.5), food concentration significantly influences metabolic activity and therefore calcification rates of *D. dianthus*. Experiments were carried out in the Chilean summer, the season when plankton concentration is enhanced within the natural environment. For cultivation of lab-corals pre-filtered seawater was used. Therefore, organisms were additionally fed twice a week with natural fjord zooplankton. This may was considerably less than *in situ* available even though the flow-through water still contained planktonic organisms to a certain degree (e.g. radiolarias, dinoflagellates, diatoms, copepod larvae...; personal observations).

Another aspect to consider is the strong variation of *in situ* replicates (Fig. 3.2). After sample preparation, corals were brought back to two different sites (*Liliguapi* and *Cross-Huinay*) within Comau fjord. Water parameters of these sites deviated: the site *Liliguapi* at the fjords mouth is exposed to more oceanic influences, *Cross-Huinay* to inner fjord conditions (Jantzen et al. submitted a). Findings may indicate growth rates as extremely variable in conjunction with habitat diversity. Nevertheless, no significant site-effect could be detected - neither for *in situ* corals nor for lab-corals in terms of origin (Jantzen et al. submitted b).

Several previous studies assessed growth rates of *D. dianthus* by means of different methodical approaches (summarized in Table 2). For the present study the buoyant weight technique was used. Results are therewith mainly comparable to outcomes of Orejas et al. (2011a). Their investigations were conducted on specimens of the Mediterranean Sea, sampled in 300 m water-depth. Corals were maintained for 250 days under controlled flow-through conditions at a temperature of 12 °C and were fed 5 times a week with Mysidacea, frozen *Cyclops* and *A. salina* nauplii. Despite higher food supply, lower growth rates of $0.06 \pm 0.03 \text{ \% d}^{-1}$ were recorded compared to the lab-growth rates of the present study.

This may rely to a certain amount on pre-adaptation to the oligotrophic Mediterranean environment (Turley 1999), again highlighting the variability of growth rates with habitat. Furthermore, corals originated from two different water depths which would in case of pre-adaptation potentially influence their metabolic rate. In contrast to the deep-dwelling Mediterranean organisms, shallow living Chilean *D. dianthus* specimens were identified to have an unusual relationship to microendolithic organisms (Försterra & Häussermann 2005). These may, with respect to symbiotic benefits in tropical corals, also deliver additional assimilates for skeletal mineralization. Nonetheless, the study of Kupprat 2011 (unpublished data) could not prove the existence of a beneficial symbiosis between *D. dianthus* and microendoliths. However, this could be explained by the small replicate number.

Another uncertainty that may have caused variances in growth rates is coral age. Several former studies observed linear extension and calcification rates of cold-water corals to decrease with increasing age (Mortensen 2001, Brooke & Young 2009, Maier et al. 2009, Gass & Roberts 2010). Individuals used in the present study varied in weight, length and diameter and were as equal as possible separated between *in situ* and lab treatments. Potential age influence on short-term growth rates was tested as part of the study of Jantzen et al. (submitted b) by comparison of coral size and amount of precipitated CaCO_3 . Neither for mass increase ($\% \text{ d}^{-1}$) nor for precipitated CaCO_3 ($\text{mg cm}^{-2} \text{ d}^{-1}$) could a correlation be obtained. This was referred to a masking by handling-effects. Corals used in the study of Orejas et al. (2011a) were 5 - 10 cm in height and 1.5 - 3 cm in diameter. However, even if size of corals was comparable between studies they do not reveal the same ages caused by the exposure to complete different environmental settings which in turn affected the growth speed.

Moreover, it has to be considered that coral growth reveals strong temporal variations. These pulsed and episodic growth events were described for temperate corals (Peirano et al. 2005), massive solitary scleractinian specimen (Nagelkerken et al. 1997) and even for cold-water corals maintained in aquaria, thus, detached from seasonal environmental influences (Mortensen 2001). This may indicate growth events to be also effected by endogenous rhythms. As already mentioned above, short-term experiments of the present study were carried out in summer which presumably triggered high growth rates at least for *in situ* corals. In case of pre-adaptation, this may also be true for corals maintained in the laboratory simply due to the relatively short time span of

acclimatization. The study of Orejas et al. (2011a) investigated *D. dianthus* over a period of 8 months. Measurements may therefore have included periods of depressed calcification activities.

It can be concluded that this study in collaboration with Jantzen et al. (submitted b) established so far the highest *in situ* growth rates of *D. dianthus* in terms of CaCO_3 precipitation. However, it has to be minded that growth rates were assessed in a short-term experiment over summer season and on shallow-living *D. dianthus* specimen. Although another study by Naumann et al. (2011) recorded similar growth rates ($0.1 - 0.3 \text{ \% d}^{-1}$) for Mediterranean *D. dianthus* specimen in cultivation, differences in methodical approaches have to be considered. Whereas Naumann et al. (2011) the total alkalinity anomalie technique applied were growth rates of the present study achieved by buoyant weighing. Methodical comparisons and constraints of these two techniques will be discussed in chapter 4.4.3.

4.4.2 Long-term calcification rates

Additionally to short-term calcification rates, the present study investigated also long-term calcification rates of *D. dianthus* (Fig. 3.3, 3.4). Purpose was to evaluate the cross-effects of nutrition and flow on the coral's growth behavior and therewith the environmental importance of these two parameters.

Chapter 4.3 describes a positive correlation between calcification rates and metabolic activity. For that reason calcification records were also found to be highest under increased food and flow conditions (HFHC treatment, Fig. 3.4).

Various studies have demonstrated the great importance of current strength for growth rates of filter feeders (e.g. Jokiel 1978, Dennison & Barnes 1988, Eckman & Duggins 1993, Fabricius et al. 1995). However, they mainly interpreted this as variations in particle flux and corresponding capture rates. As this study provided food by pipetting it onto expanded tentacles, potential flow effects can be rather applied to the thickness of the diffusive boundary layer (DBL). Thin DBL's under strong flow speeds favor the transfer of inorganic nutrients and harmful by-products between corals tissue and

surrounding water masses (Finelli et al. 2006). The calcium and bicarbonate ions needed for calcification can be therefore easily derived from the water column and delivered to the site of calcification. Shashar et al. (1993) described furthermore a negative correlation between DBL thickness and polyp size of massive corals. This could be, in regard to the present study, an explanation for the spreads of replicates. Corals within one treatment differed in length, diameter and weight assuming also natural derived differences in boundary layer thickness and associated ability for nutritional exchange. Shashar et al. (1993) predicted furthermore that small-polyped corals with thinner DBL's will be, hence, more abundant in habitats characterized by low currents. This hypothesis can be clearly supported by personal observations of *D. dianthus* distribution within Comau Fjord. The largest corals were found at the fjord's mouth (site: *Liliguapi*) where also the strongest flow speeds were detected.

Nonetheless, flow speed had no significant influence on calcification rates of *D. dianthus*. This would have been different in case of non-direct food supply. The only significant differences were detected by comparing calcification rates of the HFHC treatment with those of the starving treatments, revealing the nutritional status of greater importance for coral calcification.

Increased calcification rates with food enrichment was already shown for many zooxanthellate scleractinian corals such as *Stylophora pistillata* (Ferrier-Pagès et al. 2003, Houlbrèque et al. 2003) and *Cladocera ceaspitosa* (Rodolfo-Metalpa et al. 2008) even though symbionts provided additional amounts of energy-rich organic compounds. However, to date there has been almost no research investigating the nutritional impact on cold-water coral calcification. Exclusively the study by Naumann et al. (2011) on *D. dianthus* specimens gives first evidence that heterotrophy promotes calcification rates in scleractinian cold-water corals. They attested a rapid decline of calcification rates as a result of zooplankton exclusion (3 weeks) which suggests that *D. dianthus* allocates a high proportion of heterotrophic energy to calcification processes. Even though starved specimens of the present study were fed once a week at least to cover their respiratory carbon loss, substantial declines of calcification rates were also visible within the first month during acclimatization (Fig. 3.3). This decline continued in the second month only for starving specimen under high flow conditions (LFHC). The longer time span needed for acclimatization can be a further explanation for low growth rates of respective treatment. A positive trend of calcification rates throughout all treatments emerged

exclusively for the last month (Fig. 3.3), implying entire acclimatization to the experimental setup. An increase in calcification rates was even observed for starving specimens. This contradiction can be explained by two different issues. One possible explanation could be the utilization of other energy-sources such as tissue biomass (Barnes & Lough 1993) or dissolved organic matter (Grover et al. 2008) which may serve to sustain calcification during low food periods. It is assumable that respective metabolic pathways get only activated when corals were exposed to low food conditions for a certain time. This was also hypothesized by Form & Riebesell (2011) regarding adaptation of *L. pertusa* to aragonite sub-saturated waters (Ω_{Ar}). Another possible explanation may be inner-annual growth rhythms. Growth bandings of deep-dwelling *D. dianthus* specimens were normally found to be correlated with seasonal variations of food supply (Cheng et al. 2000, Adkins et al. 2004). Nevertheless, Mortensen (2001) described episodic growth rhythms also for cold-water corals in cultivation. Rapid growth events were followed by longer time intervals (> 4 weeks) without growth.

In comparison to the laboratory short-term growth rates ($0.09 \pm 0.08 \text{ \% d}^{-1}$) it is obvious that even the highest growth rates of the long-term experiment ($0.07 \pm 0.05 \text{ \% d}^{-1}$) stayed below. These depressed calcification rates may be stress response to additional handling-effects which corals experienced after conduction of the short-term experiment (freight to Bremerhaven, acclimatized to a new aquarium system, arranged in a first setup of flow-chambers which was finally ceased, re-arranged in a second setup). Already Brown & Howard (1985) found corals to respond to stress events by changes in metabolism and growth rates. The scleractinian warm-water coral *Porites porites* for instance invested less energy to tissue growth under enhanced sedimentation rates (Edmunds & Davies 1988). Also the cold-water coral *L. pertusa* shows a short-term decline of calcification when pH decreases about 0.15 units (Form & Riebesell 2011). Brooke & Young (2009) expect furthermore that cold-water corals just maintain skeletal structure without additional growth when exposed to suboptimal conditions.

The conditions within the aquarium system were tried to resemble *in situ* properties found at the sampling sites. Water was maintained to a constant temperature of $\sim 12 \text{ }^{\circ}\text{C}$ which corresponds to the temperature of the coral's natural habitat. Even though corals were not maintained in flow-through facilities as during the short-term experiment, they were reared in re-circulating 350 L aquarium systems with a weekly exchange rate of \sim

10 %. Therefore, unfiltered North Sea water was used in order to provide a natural ratio of nutrients and trace elements. Nonetheless, the average nutrient concentrations of water masses were higher as under natural conditions (Silva 2008) which still seem to be in range of tolerance when considering the positive trend in calcification rates to end of the long-term experiment. Extension of the long-term experiment might have even lead to calcification rates in a comparable range to those of the short-term experiment, since the standard deviations already overlapped. Based on those considerations it can be concluded that corals situated within the HFHC (Fig. 3.4) treatment do not only in comparison to the other treatments face favorable conditions but rather can also be compared to treatments of the short-term experiment conducted under close to *in situ* conditions (water parameters, food spectrum,...).

Also maintenance effects in general could be a potential reason for a successive decline of growth rates with time. Corals used for the long-term experiment were sampled on expeditions in 2010 and 2011. Although these corals were consistently distributed throughout the treatments, individuals of 2010 could have bias the average. Nevertheless, coral growth and residence time within maintenance facilities has not been checked for correlation since observant monitoring showed no differences in coral behavior and activity.

In summary, received results support the previous assumption that calcification rates of cold-water corals are significantly influenced by their nutritional status. Well-nourished corals are able to invest more energy for the highly consumptive processes of calcification. Furthermore, findings provide some evidence that *D. dianthus* can meet energy demands also by uptake of other food sources (e.g. dissolved organic matter) during periods of low food.

4.4.3 Methodological considerations

Discussed calcification rates exclusively base on results obtained via the buoyancy weight technique. The total alkalinity (TA) anomaly technique was ceased during the

course of the time-series since pre-experiments at field station *Fundación Huinay* and first results of the long-term experiment ranged within the limit of analytical precision ($\sim 10 \mu\text{M}$, calculated from seawater standards). In the following, potential reasons will be discussed.

The TA technique is based on the assumption of a 2 molar decrease in TA for every mol CaCO_3 precipitated (Smith & Key 1975). Besides calcification and dissolution processes also nutrient fluxes such as sulfate reduction (Berner et al. 1970) or uptake and release of PO_4^{3-} , NH_3^- and NH_4^+ (Brewer & Goldman 1976) were found to alter TA. The present study determined a net release of dissolved nutrients during incubations (varies with coral size, metabolic activity and treatment) which was for *D. dianthus* already shown by Naumann et al. (2011) with respect to total organic carbon ($0.21 - 0.59 \mu\text{mol TOC cm}^{-2} \text{d}^{-1}$). Even though TA values were corrected for changes in nutrient concentrations (see equation 10) also the excretion of undetectable organic acids could have effect TA measurements to an unknown degree (Langdon et al. 2010).

Incubations were carried out either with fleece filtered fjord water on-site or unfiltered North Sea water during the long-term time series. This implies also large amounts of residual microorganisms within the incubation medium. Maier et al. (2011) found dissolved nutrient excretions during incubations of cold-water corals (*L. pertusa* and *M. oculata*) to positively affect bacterial growth. Hence, the biological activity of microorganisms seems necessary to consider. The study of Naumann et al. (2011) supports this assumption. Their incubation experiments were carried out with $50 \mu\text{m}$ pre-filtered seawater and revealed *D. dianthus* calcification rates of $0.1 - 0.3 \%$ per day. Incubation time (6 h) and temperature ($12.0 \pm 0.1^\circ\text{C}$) were equal to those of the present study which may indicate pre-filtration as an important parameter influencing successful calcification measurements. Nevertheless, it has to be kept in mind that Mediterranean *D. dianthus* specimens (Naumann et al. 2011) lack infestation by microendolithic organisms. Even though the present thesis conducted incubation experiments under low light levels, photosynthetic processes of endoliths may have altered TA additionally (Brewer & Goldman 1976).

Moreover, Mediterranean specimens were incubated in a volume of 240 mL (compared to 310 and 706 mL of the present study) which assuredly supported adequate calcification signals. To obtain reasonable calcification signals, changes in TA need to be 10 – 30 times higher than the analytical precision (Langdon et al. 2010). Calcification

signals can be intensified by either adjusting the incubation time and/or the water volume the organism is kept in. Neither an extension of the incubation time, which would have lead to enhanced oxygen depletion and excretion of harmful by-products, nor a smaller incubation volume, simply due to organism sizes, could be applied in the present study to optimize signal power.

Another aspect to consider is the general growth behavior of investigated corals. Detected growth rates of Naumann et al. (2011) are comparable to *in situ* growth rates of the present study obtained via buoyant weighing. However, these results should be compared with caution due to the different methodological approaches. Whereas the buoyancy weight technique examines the calcifying organism itself, the TA technique analyses the incubation water the organism was in exchange with. This non-direct technique is thus sensitive also to other biological processes occurring within the water column. The lab-corals used in the present study for the TA technique were proved via buoyant weighing to grow significantly lower than corals *in situ* (Fig. 3.2). These general low calcification rates ($0.05 \pm 0.03 \text{ \% day}^{-1}$) might have been masked by the methodical error.

In conclusion, the biological activity of microorganisms may be considered as a main reason for alteration of TA values in the present study. Calcification measurements via the TA technique can be, hence, in future studies partly be improved by pre-filtration of the incubation medium.

5 Conclusion

Results confirm previous assumptions; the most favorable conditions for growth and metabolic fitness of *D. dianthus* were obtained under exposure to high food concentrations and flow velocities. The food intake significantly influenced key physiological processes of *D. dianthus* and was furthermore found to be flow-dependent. Capture rates were highest under moderate flow regimes with declining trends towards stagnant conditions and increased flow speeds. By supply of their natural food spectrum, also the diet of *D. dianthus* was analyzed. Approximately half of ingested food consisted of prey items greater 1000 μm .

Nevertheless, transferring laboratory results to *in situ* conditions should be done with caution due to different environmental settings. This is also supported by findings of the short-term experiment which shows significantly lower growth rates of corals maintained in the laboratory as of corals grown *in situ*. In general, this study investigated highest *in situ* calcification rates recorded so far for *D. dianthus*, even comparable to rates of some slow growing warm-water corals. These high growth rates may let assume shallow *D. dianthus* specimen to have relatively short recovery times after disturbances of either natural or anthropogenic origin. Moreover, it became evident that *D. dianthus* is a robust and unsusceptible organism considering the high acclimatization potential observed within a time span of 3 month. They almost recovered from the intensive handling procedure and even tolerated enhanced nutrient concentrations within the aquaria water, when compared to the records of Comau Fjord. This might be the result of pre-adaptation to a highly variable environment and could be an advantage in terms of supposed nutrient pollution caused by the expanding aquaculture in these fjord regions.

Short recovery times and high acclimatization potentials of *D. dianthus* would also have positive effects for the whole ecosystem. *Desmophyllum dianthus* is the dominant scleractinian coral within the austral Chilean fjord region and their formation of extensive coral banks provides structural habitat to a diverse benthic fjord community. Decline of these unique coral communities would have unforeseeable consequences for an ecosystem which is by now only rudimentarily explored and understood.

The present thesis broadened the current knowledge about these shallow-living cold-water corals by investigating their feeding ecology, metabolic fitness and growth behavior under varied environmental parameters. Findings contribute to debated future scenarios of respective shallow cold-water coral ecosystems and can be used for predictions of organism responses and population dynamics.

Further investigations on *D. dianthus* are needed to comprehend the complexity and ecological relevance of these ecosystems, the triggers for their unusual shallow occurrence and their role in a changing environment. Studies should encompass subsequent approaches on growth, fitness and feeding as well as examinations of reproduction, dispersal, settlement and defense mechanisms. Particularly the resilience towards future predicted changes in temperature and pH would be necessary to consider. Appropriated experiments carried out *in situ* among their natural ecoenvironmental gradients would deliver most reliable results.

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Appendix

Appendix 1: Statistical reports of feeding experiments, for total capture rates (A1), percentage capture rates (A2 – A3) and species selectivity (A4)

Table A1: Feeding experiment statistics (Fig. 6A). Mann-Whitney-U test ($p < 0.05$ = significant) was performed on total capture rates (prey $\text{cm}^{-2} \text{h}^{-1}$) of *D. dianthus* within each flow treatment (0 cm s^{-1} , 2 cm s^{-1} , 5 cm s^{-1}) with $n=4$.

Flow Speed	0 cm s^{-1}	2 cm s^{-1}
2 cm s^{-1}	0.428	-
5 cm s^{-1}	0.792	0.093

Table A2: Feeding experiment statistics (Fig. 6B). Different prey size classes ($> 1000 \mu\text{m}$, $500 - 1000 \mu\text{m}$, $< 500 \mu\text{m}$) within each single flow treatment (0 cm s^{-1} , 2 cm s^{-1} , 5 cm s^{-1}) were statistically (Mann-Whitney-U test) compared. Results (p-values) base on size fractioned total capture rates (prey $\text{cm}^{-2} \text{h}^{-1}$) of *D. dianthus*. Each treatment consists of 4 replicates.

	0 cm s^{-1}		2 cm s^{-1}		5 cm s^{-1}	
	$>1000\mu\text{m}$	$500-1000 \mu\text{m}$	$>1000\mu\text{m}$	$500-1000 \mu\text{m}$	$>1000\mu\text{m}$	$500-1000 \mu\text{m}$
$500-1000 \mu\text{m}$	0.151	-	0.132	-	0.588	-
$< 500\mu\text{m}$	0.310	0.547	0.309	0.937	0.310	0.485

Table A3: Feeding experiment statistics (Fig. 6B). Same prey size classes ($> 1000 \mu\text{m}$, $500 - 1000 \mu\text{m}$, $< 500 \mu\text{m}$) of different flow treatments (0 cm s^{-1} , 2 cm s^{-1} , 5 cm s^{-1}) were statistically (Mann-Whitney-U test) compared. Results (p-values) base on size fractioned total capture rates (prey $\text{cm}^{-2} \text{h}^{-1}$) of *D. dianthus*. Each treatment consists of 4 replicates.

Prey Size Class	Flow Speed	0 cm s ⁻¹	2cm s ⁻¹
>1000 µm	2cm s ⁻¹	0.931	-
	5cm s ⁻¹	0.329	0.240
500-1000 µm	2cm s ⁻¹	0.662	-
	5cm s ⁻¹	0.931	0.937
<500 µm	2cm s ⁻¹	0.429	-
	5cm s ⁻¹	0.537	0.699

Table A4: Prey selection of *D. dianthus* shown on species level, using Ivlev's Index (E). E ranges from +1 to -1 with 0 representing "no selection"; comparative illustrated for size fractioned samples (> 1000 µm, 500 - 1000 µm, < 500 µm) of different flow treatments (0 cm s⁻¹, 1.8 cm s⁻¹, 4.3 cm s⁻¹).

Size Class Flow (cm s ⁻¹)	> 1000 µm			500 - 1000µm			< 500µm		
	0	1.8	4.3	0	1.8	4.3	0	1.8	4.3
Euphausia	0.29	0.18	0.4	-	-	-	-	-	-
Chaetognatha	-0.06	0.16	0.10	-0.30	-0.02	1.00	-	-	-
Siphonophora	-0.19	-0.27	-0.15	-0.98	0.44	0.25	-	-	-
Rhincalanus	-0.40	0.25	0.12	-0.63	-0.22	0.87	-	-	-
Euchaeta	0.00	0.20	-0.10	-0.03	-0.05	0.31	-	-	-
Paracalanus	-0.02	-0.58	-0.35	-0.07	-0.30	0.21	-	-	-
Calanus	0.08	0.65	-0.01	-0.41	-0.63	0.12	-	-	-
Metridia	-	-	-	-0.15	-0.13	0.30	0.11	-0.08	0.23
Oithona	-	-	-	-	-	-	0.10	-1.00	-0.56
Cirripedia Nauplii	-	-	-	-0.27	-0.34	0.01	-0.17	-0.62	-0.26
Polychaete Larvea	-	-	-	-0.2	-0.13	0.47	0.26	-0.04	-0.34
Echinoderm Larvea	-	-	-	-0.14	-0.09	0.12	-0.11	-0.14	0.16
Bryozoa Larvea	-	-	-	0.01	-0.18	0.01	-0.10	-0.29	0.20
Ostracoda	-	-	-	-0.24	0.14	0.39	0.28	0.32	0.44
Cladocera	-	-	-	0.11	0.07	0.24	0.27	-0.81	-0.07
Decapoda Larvea	-	-	-	-0.04	0.53	0.57	-0.41	-0.07	0.31
Copepod Nauplii	-	-	-	0.01	1.00	0.37	0.03	0.12	0.01

Appendix 2: Data of short-term and long-term calcification rates

Short-term growth rates			Long-term growth rates		
Treatment	Coral ID	mg CaCO ₃ cm ⁻² d ⁻¹	Treatment	Coral ID	mg CaCO ₃ cm ⁻² d ⁻¹
<i>In situ</i>	(L) 0	5,94	LFLC	7L	0,65102
<i>In situ</i>	(L) 1	6,06	LFLC	XE1	0,53653
<i>In situ</i>	(L) 2	2,84	LFLC	SW4	0,46990
<i>In situ</i>	(L) 3	5,69	LFLC	1x	0,86276
<i>In situ</i>	(L) 5	4,67	LFLC	Li8	0,35270
<i>In situ</i>	(L) 6	0,87	LFLC	3x	0,99747
<i>In situ</i>	(L) 7	7,57	LFLC	PG2	1,51063
<i>In situ</i>	(L) 8	3,04	LFLC	SW5	0,30873
<i>In situ</i>	(L) 9	6,87	LFLC	o.l.gr.	-0,01403
<i>In situ</i>	X0	8,69			
<i>In situ</i>	X1	3,06	LFHC	6L	0,6491
<i>In situ</i>	X2	11,14	LFHC	KI Li8	0,2159
<i>In situ</i>	X3	3,04	LFHC	6x	0,3506
<i>In situ</i>	X4	1,35	LFHC	PG5	0,0964
<i>In situ</i>	X5	14,48	LFHC	Li10	-0,0037
<i>In situ</i>	X6	2,99	LFHC	2L	0,6675
<i>In situ</i>	X7	7,78	LFHC	7x	1,5170
<i>In situ</i>	X8	4,72	LFHC	XE5	-0,1487
<i>In situ</i>	X9	2,58	LFHC	SW1	-0,6666
Laboratory	L1	1,5996	HFLC	Li6	0,2180
Laboratory	L2	1,8595	HFLC	PG7	0,5923
Laboratory	L3	5,4964	HFLC	X1	1,3757
Laboratory	L4	2,3978	HFLC	0X	1,0383
Laboratory	L5	0,5546	HFLC	3L	2,4706
Laboratory	L6	1,5973	HFLC	4L	1,2600
Laboratory	L7	1,0484	HFLC	PG6	0,0871
Laboratory	L8	1,0805	HFLC	SW6	0,7941
Laboratory	X1	0,9337	HFLC	o.l.kl.	2,4761
Laboratory	X2	1,9870			
Laboratory	X3	3,7135	HFHC	9L	0,6704
Laboratory	X4	1,8653	HFHC	5x	1,7377
Laboratory	X5	2,4324	HFHC	KL X5	2,0149
Laboratory	X6	3,5636	HFHC	X4	1,6594
Laboratory	X7	1,9079	HFHC	1L	1,0810
Laboratory	X8	3,3854	HFHC	4x	3,3389
Laboratory	X9	0,3805	HFHC	SW2	1,2654
			HFHC	8X	0,8135
			HFHC	x5	2,8176

Appendix 3: Measured mass increase of each coral for the three time intervals of the long-term experiment

Treatment	Coral ID	Total Mass Increase (mg CaCO ₃ cm ⁻²)		
		t0 - t1	t1 -t2	t2 -t3
LFLC	7L	18,02	1,89	33,914
LFLC	XE1	14,96	9,96	19,552
LFLC	SW4	1,60	0,81	25,034
LFLC	1x	32,52	28,81	18,643
LFLC	Li8	59,47	16,82	2,582
LFLC	3x	26,25	17,23	37,626
LFLC	PG2	43,15	32,18	50,905
LFLC	SW5	7,34	3,59	13,395
LFLC	o.l.gr.	10,23	5,65	-6,425
LFHC	6L	1,64	0,26	35,440
LFHC	Kl Li8	-28,52	-27,38	39,257
LFHC	6x	-45,47	-133,15	152,433
LFHC	PG5	-17,51	-9,19	14,495
LFHC	Li10	-5,39	-1,39	1,187
LFHC	2L	-0,63	-0,22	36,927
LFHC	7x	2,46	2,16	81,270
LFHC	XE5	-15,58	-6,36	-1,818
LFHC	SW1	-36,42	-16,72	-19,940
HFLC	Li6	-17,75	28,07	-16,081
HFLC	PG7	-6,74	5,13	27,447
HFLC	X1	-6,87	27,77	47,892
HFLC	0X	13,63	16,64	40,465
HFLC	3L	-85,42	103,31	32,574
HFLC	4L	6,62	27,76	41,538
HFLC	PG6	-20,02	-2,68	7,467
HFLC	SW6	-6,25	11,85	31,830
HFLC	o.l.kl.	-11,62	27,62	108,564
HFHC	9L	19,89	-4,30	41,176
HFHC	5x	23,08	36,03	59,546
HFHC	KL X5	6,96	49,37	61,448
HFHC	X4	-10,74	38,43	52,839
HFHC	1L	-4,82	21,35	38,103
HFHC	4x	18,41	78,95	104,684
HFHC	SW2	-11,51	26,94	42,653
HFHC	8X	-7,30	11,29	33,452
HFHC	x5	1,33	58,63	96,336

Appendix 4: Statistical report for data analysis of long-term calcification rates**One Way Analysis of Variance****Data source:** Data 1 in Notebook1.JNB**Normality Test:** Passed (P = 0,057)**Equal Variance Test:** Passed (P = 0,226)

Group Name	N	Missing	Mean	Std Dev	SEM
LFLC	9	0	0,631	0,446	0,149
LFHC	9	0	0,297	0,614	0,205
HFLC	9	0	1,146	0,867	0,289
HFHC	9	0	1,711	0,898	0,299

Source of Variation	DF	SS	MS	F	P
Between Groups	3	10,306	3,435	6,437	0,002
Residual	32	17,079	0,534		
Total	35	27,386			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0,002).

Power of performed test with alpha = 0,050: 0,914

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0,05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
HFHC vs. LFHC	1,413	4,104	<0,001	0,009	Yes
HFHC vs. LFLC	1,080	3,137	0,004	0,010	Yes
HFLC vs. LFHC	0,848	2,463	0,019	0,013	No
HFHC vs. HFLC	0,565	1,641	0,111	0,017	No
HFLC vs. LFLC	0,515	1,496	0,144	0,025	No
LFLC vs. LFHC	0,333	0,967	0,341	0,050	No

Two Way Analysis of Variance

Data source: Data 1 in Notebook1.JNB

General Linear Model (No Interactions)

Dependent Variable: Col 6

Normality Test: Passed (P = 0,197)

Equal Variance Test: Passed (P = 1,000)

Source of Variation	DF	SS	MS	F	P
food status	1	0,0132	0,0132	1,594	0,426
flow	1	0,00104	0,00104	0,126	0,783
Residual	1	0,00826	0,00826		
Total	3	0,0225	0,00749		

The difference in the mean values among the different levels of food status is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in flow. There is not a statistically significant difference (P = 0,426).

The difference in the mean values among the different levels of flow is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in food status. There is not a statistically significant difference (P = 0,783).

Power of performed test with alpha = 0,0500: for food status : 0,104

Power of performed test with alpha = 0,0500: for flow : 0,0926

Least square means for food status :

Group Mean

low 0,0775

high 0,192

Std Err of LS Mean = 0,0643

Least square means for flow :

Group Mean

2,000 0,119

5,000 0,151

Std Err of LS Mean = 0,0643

Appendix 5: Changes in respiration rate during long-term experiment

Δ Respiration Rates (t1 - t3)		
Treatment	Coral ID	mg O₂ cm⁻² h⁻¹
LFLC	7L	-0,00052
LFLC	XE1	-0,00220
LFLC	SW4	-0,00416
LFLC	1x	-0,00535
LFLC	Li8	0,00370
LFLC	3x	-0,01164
LFLC	PG2	-0,00049
LFLC	SW5	0,00054
LFLC	o.l.gr.	-0,00442
LFHC	6L	-0,0016
LFHC	KI Li8	0,0014
LFHC	6x	-0,0028
LFHC	PG5	-0,0027
LFHC	Li10	-0,0077
LFHC	2L	0,0015
LFHC	7x	0,0054
LFHC	XE5	-0,0006
LFHC	SW1	-0,0031
HFLC	Li6	-0,0032
HFLC	PG7	-0,0020
HFLC	X1	-0,0091
HFLC	0X	0,0027
HFLC	3L	0,000
HFLC	4L	0,009
HFLC	PG6	0,000
HFLC	SW6	0,003
HFLC	o.l.kl.	0,030
HFHC	9L	0,003
HFHC	5x	0,005
HFHC	KL X5	0,007
HFHC	X4	0,006
HFHC	1L	0,004
HFHC	4x	-0,008
HFHC	SW2	0,010
HFHC	8x	0,166
HFHC	x5	0,003

Appendix 6: Statistical report for data analysis of long-term changes in metabolic activity

One Way Analysis of Variance

Normality Test: Failed ($P < 0,050$)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
LFLC	9	0	-0,00152	-0,00959	0,00109
LFHC	9	0	-0,000802	-0,00540	0,00283
HFLC	9	0	0,00355	0,00190	0,00814
HFHC	9	0	0,0120	0,00674	0,0159

$H = 15,340$ with 3 degrees of freedom. ($P = 0,002$)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = 0,002$)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0,05
HFHC vs LFLC	157,000	4,967	Yes
HFHC vs LFHC	123,000	3,892	Yes
HFHC vs HFLC	46,000	1,455	No
HFLC vs LFLC	111,000	3,512	No
HFLC vs LFHC	77,000	2,436	Do Not Test
LFHC vs LFLC	34,000	1,076	Do Not Test

Two Way Analysis of Variance

General Linear Model (No Interactions)

Dependent Variable: metabolic activity

Normality Test: Passed (P = 0,197)

Equal Variance Test: Passed (P = 1,000)

Source of Variation	DF	SS	MS	F	P
food status	1	0,000420	0,000420	3,812	0,301
flow	1	0,000132	0,000132	1,200	0,471
Residual	1	0,000110	0,000110		
Total	3	0,000663	0,000221		

The difference in the mean values among the different levels of food status is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in flow. There is not a statistically significant difference (P = 0,301).

The difference in the mean values among the different levels of flow is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in food status. There is not a statistically significant difference (P = 0,471).

Power of performed test with alpha = 0,0500: for food status : 0,139

Power of performed test with alpha = 0,0500: for flow : 0,0965

Least square means for food status :

Group	Mean
low	-0,00350
high	0,0170

Std Err of LS Mean = 0,00742

Least square means for flow :

Group	Mean
2,000	0,001000
5,000	0,0125

Std Err of LS Mean = 0,00742

Appendix 7: Statistical report for the Linear Regression analyses applied for mass increase and respiration rate

Linear Regression

Data source: Data 1 in Notebook1

Col 2 = 0,0103 + (0,000179 * Col 1)

N = 35

R = 0,647 Rsqr = 0,418 Adj Rsqr = 0,401

Standard Error of Estimate = 0,008

	Coefficient	Std. Error	t	P
Constant	0,0103	0,00193	5,323	<0,001
Col 1	0,000179	0,0000367	4,871	<0,001

Analysis of Variance:

	DF	SS	MS	F	P
Regression	1	0,00143	0,00143	23,724	<0,001
Residual	33	0,00199	0,0000604		
Total	34	0,00343	0,000101		

Normality Test (Shapiro-Wilk) Passed (P = 0,136)

Constant Variance Test: Passed (P = 0,091)

Power of performed test with alpha = 0,050: 0,992

Appendix 8: Illustration of natural zooplankton species used for feeding experiments
(Detailed information on page 90)

A side effect of this work was the first description of zooplankton assemblages within Comau Fjord for the time interval between February and March. The species diversity equaled those of the adjacent Gulf of Ancud.

a)



b)



c)



d)



e)



f)



g)



h)



i)



j)



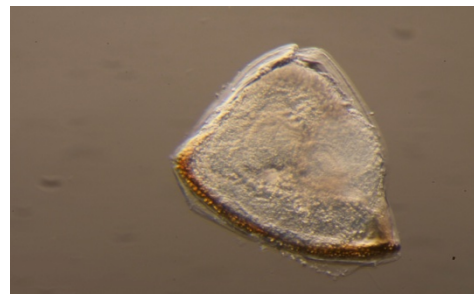
k)



l)



m)

**a-d) Copepoda**

- | | |
|--------------------|--------------------------|
| a) Rhincalanus sp. | size: 2400 μm |
| b) Paracalanus sp. | size: 1300 μm |
| c) Oithona sp. | size: 430 μm |
| d) Euchaeta sp. | size: 1800 μm |

e) Euphausia sp. size: 3200 μm

f) Ostracoda sp. size: 750 μm

g) Decapoda larvae (Zoëa) size: 620 μm

h) Cirripedia nauplii size: 310 μm

i) Echinodermata larvae size: 890 μm

j) Polychaeta larvae size: 3300 μm

k) Chaetognatha size: > 1000 μm

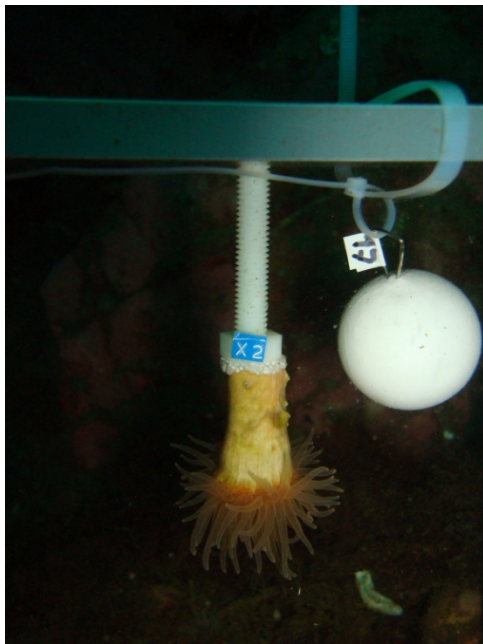
l) Mesusae size: 4800 μm

m) Cladocera sp. size: 370 μm

Appendix 9: Supplementary Photographs



Close-up of *D. dianthus* aggregation with associated benthic fauna (C. Jantzen)



Prepared coral fixed to customized holder with gypsum plaster for flow measurements



Aquaria facilities at AWI

**Erklärung gemäß § 21 der Diplomprüfungsordnung,
Christian-Albrechts-Universität zu Kiel**

Hiermit erkläre ich, Stefanie Sokol, die vorliegende Arbeit mit dem Titel „Influence of heterotrophy and flow on calcification of the cold-water coral *Desmophyllum dianthus*“ selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben.

Weiterhin versichere ich, dass diese Arbeit zu keiner Zeit als Abschlussarbeit an anderer Stelle vorgelegen hat.

Ort, Datum

Signatur